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**PhD Course in Life Sciences and Biotechnologies**

# **CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF USUTU VIRUS ISOLATES IN SARDINIA**

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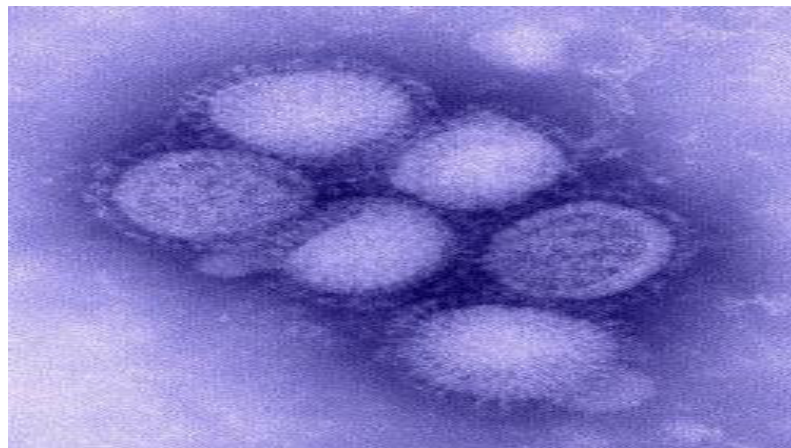
## Chapter 1

### 1.1 Classification of *Usutu virus* (USUV)

*Usutu virus* (USUV) (**fig. 1.1**) is an African mosquito-borne flavivirus, belonging to the *Japanese encephalitis virus* (JEV) serocomplex.

USUV was originally isolated from mosquitoes (*C. Neavei*) collected in Ndumu, Natal, South Africa in 1959 and named after the river homonymous (Usutu river) where it was found. It is one of the most neglected Old World encephalic flaviviruses.

USUV is a zoonosis and, like other Flavivirus, is able to propagate both in Arthropods and in Vertebrates, including Humans.



**Fig. 1.1** *Usutu virus* (from Steinmetz HW *et al.* 2010)

USUV is classified in the group of *Arbovirus*, family *Flaviviridae*, genus *Flavivirus* and the type specie is the *Yellow fever virus* (YFV).

The genus *Flavivirus*, of the *Flaviviridae* family, is composed of more than 70 viruses. Among them we can find the *Japanese encephalitis virus* (JEV), *West Nile virus* (WNV),

*Murray Valley* encephalitis virus (MVEV), *Dengue* virus (DENV) and *Yellow fever* virus (YFV) which are all extremely dangerous to human health. (Karabatsos N. 1985; Kuno *et al.* 1998; Poidinger *et al.* 1996).

In addition, USUV is closely related to several human and animal pathogenic members of this family including WNV, MVEV and JEV (Calisher CH *et al.* 2003).

We can classify the members of the genus *Flavivirus* in two different ways:

- Based on cross-neutralization studies, they have been divided into 8 antigenic complex (**table 1.1**) (Calisher CH *et al.* 1989);

Antigenic complex	Viruses
Tick-borne encephalitis	(Russian spring–summer encephalitis, Central European encephalitis), Omsk haemorrhagic fever, louping ill, Kyasanur Forest disease, (Langat, Phnom Penh bat, Carey Island), Negishi, Powassan, Karshi, Royal Farm
Rio Bravo	Rio Bravo, Entebbe bat, Dakar bat, Bukalasa bat, Saboya, Apoi
Japanese encephalitis	Japanese encephalitis, Murray Valley encephalitis, Kokobera, Alfuy, Stratford, St Louis encephalitis, Usutu, West Nile, Kunjin, Koutango
Tyulenyi	Tyulenyi, Saumarez Reef, Meaban
Ntaya	Ntaya, (Tembusu, Yokose), (Israel turkey meningoencephalitis, Bagaza)
Uganda S	Uganda S, Banzi, Bouboui, Edge Hill
Dengue	Dengue 1, dengue 2, dengue 3, dengue 4
Modoc	Modoc, Cowbone Ridge, Jutiapa, Sal Vieja, San Perlita

\* Viruses more closely related to each other than to others of the antigenic complex are in parentheses.

**Table 1.1 Antigenic serocomplex of Flavivirus** (from Calisher CH *et al.* 1989)

Most of these viruses are serologically classified into 8 antigenic serocomplex, however 17 others viruses are not sufficiently related to each other to include them in any of these complexes.

USUV belongs to the JEV serocomplex.

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- Based on phylogenetic analysis carried out on partial nucleotide sequences of the NS5 coding and 3' noncoding regions (Kuno *et al.* 1998).

Virus <sup>a</sup>	Clade	Antigenic complex
<b>Non-vector cluster</b>		
CFA		
Apoi		
<b>San Perlita; Jutiapa</b>	I	Modoc
Montana myotis leukoencephalitis; Modoc; Cowbone Ridge; Sal Vieja	II	Modoc
<b>Bukalasa bat; Dakar bat; Rio Bravo; Carey Island; Phnom Penh bat; Batu Cave</b>	III	Rio Bravo
<b>Tick-borne cluster</b>		
Gadgets Gully; Royal Farm; Pow; Karshi; KFD; Langat; Omsk HF;	IV	TBE
TBE-far eastern subtype; RSSE; TBE-CE; Negishi		
<b>Kadam; Tyuleniy; Saumarez Reef; Meaban</b>	V	Tyuleniy
<b>Mosquito-borne cluster</b>		
Edge Hill; Bouboui; Uganda S; Banzi; Jugra; Saboya; Potiskum	VI	Uganda S
Sepik; YF	VII	
Sokuluk; Entebbe bat; Yokose	VIII	
<b>DEN-1 to -4</b>	IX	DEN
Kedougou		
Zika; Spondweni	X	
SLE; Rocio; Ilheus; Tembusu; THCAr; Ntaya; Israel turkey meningoencephalitis; Bagaza	XI	Ntaya
Naranjal; Bussuquara; Aroa; Iguape	XII	
<b>Kokobera; Stratford</b>	XIII	JE
<b>Cacipacore; Yaounde; Koutango; Kunjin; WN; Alfuy; JE; Murray Valley encephalitis; Usutu</b>	XIV	JE

<sup>a</sup> The viruses in boldface belong to the corresponding antigenic complex to the right.

**Table 1.2 Assignment of *Flaviviruses* to clusters and clades (from Kuno *et al.* 1998)**

If we analyse the genetic features of the *Flavivirus* genus, it is segregated into 14 clades belonging to 3 different clusters. Each cluster is then divided in different clades, as shown in the table (1.2) above.

USUV is classified in the JEV group of the mosquito-borne cluster, together with *Cacipacore* virus (CPCV), *Koutango* virus (KOUV), JEV, MVEV, *Alfuy* virus (ALFV), *St Louis* encephalitis virus (SLEV), WNV, *Kunjin* virus (KUNV), and *Yaounde* virus (YAOV) (Heinz *et al.* 2000).

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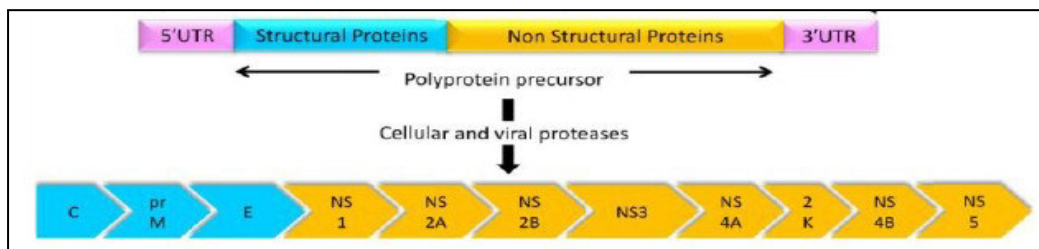
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## 1.2 Molecular features of USUV

Similar to other flaviviruses, USUV is a spherical, small-enveloped virus with a diameter 40-60 nm. The virion comprises three structural proteins, the capsid (C), the membrane (M), and the envelope (E) proteins and an 11 kb single-stranded, positive sense RNA genome (Rice *et al.* 1986). The genomic RNA (**fig 1.2**) contains a 5' cap structure, but lacks a polyadenylated tail. It acts as mRNA for translation of a single open reading frame (ORF) encoding the viral proteins as a large polyprotein that is co- and post- translational processed by cellular and viral proteases into at least 10 separate products. The N-terminal region encodes the structural proteins C, pre-M, and E, followed by the nonstructural proteins NS1 (soluble complement-fixing antigen), NS2A and NS2B, NS3 (serine protease/RNA helicase), NS4A, 2K, NS4B, and NS5 (RNA dependent RNA polymerase/methyltransferase) (Rice *et al.* 1985).



**Fig. 1.2** USUV: its gene structure and the proteins encoded by its genome.

The polyprotein precursor is cleaved by cellular and viral proteases to yield three structural proteins C (97-474 nt), prM (475-975 nt), and E (976-2475 nt) and eight non-structural proteins NS1 (2476-3531 nt), NS2A (3532-4212 nt), NS2B (4213-4605 nt), NS3 (4604-6462 nt), NS4A (6463-6840 nt), 2K (6841-6909 nt), NS4B (6910-7683 nt), and NS5 (7684-10398 nt) (from Ashraf *et al.* 2015).

At the 5'-terminal there is a UTR which is long about 100 nt and, at the 3'-terminal, is usually long about 670 nt.

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It is noticeable how the UTRs play a crucial role in the *Flavivirus*, not only for the initiation and for the regulation of the translation but also for the replication and the virion assembly. In particular the 3'-UTRs of various flaviviruses demonstrate extensive heterogeneity in size and sequence of their proximal parts where long deletions, insertions, sequence repeats and even poly (A) stretches have been observed (Mandl *et al.* 1993; Wallner *et al.* 1995; Wang *et al.* 1996; Gritsun *et al.* 1997). In contrast, the distal part of the 3'-UTR (330–400 nucleotides in length) exhibits relatively high sequence identity and was previously defined as the functional 'core' element of the flaviviral 3'-UTR (Wallner *et al.* 1995).

The functions of the 3'-UTR can be mediated through signal sequences and elements of RNA secondary structure. Indeed, a number of conserved sequence motifs have been identified in the 3'-UTR of flaviviruses (Hahn *et al.* 1987; Mandl *et al.* 1993), although there is no direct evidence for their function.

On the other hand, a region of about 100 nt at the 3'-terminal of all flaviviruses is known to form a "long stable hairpin structure" (3'-LSH) which shows conformational conservation despite differences in sequence. This would suggest that the 3'- LSH is under selective constraint and, therefore, of functional importance (Grange *et al.* 1985; Brinton *et al.* 1986; Hahn *et al.* 1987; Mohan and Padmanabhan, 1991; Mandl *et al.* 1993; Shi *et al.* 1996). The 3'-LSH can exhibit a specific interaction with host cellular proteins (Blackwell and Brinton, 1995, 1997) and viral polymerase (Chen *et al.* 1997), which points to the direct involvement of this structure in the initiation of viral minus strand RNA synthesis (Proutsky *et al.* 1999).

In 2004, with the use of phylogenetic analyses to examine the whole genome sequence of USUV, it was established the inclusion of one African (SouthAfrica-1959) and one European (Vienna-2001) lineage of USUV (**fig.1.3**) (Bakonyi *et al.* 2004). These two strains

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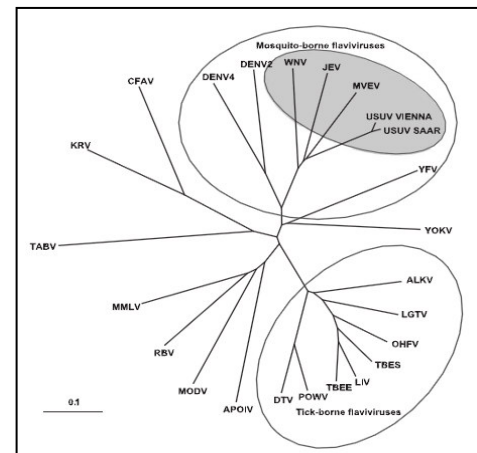
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are 97% and 99% identical at nucleotide and amino acid level, respectively (Bakonyi *et al.* 2004).

**Fig.1.3** Phylogenetic tree illustrating the genetic relationship between flaviviruses based on their complete genome sequences. (from Bakonyi *et al.* 2004)



A research was published in 2016 which established that USUV can be classified into six distinct lineages and that the most recent common ancestor of the recent European epizootics emerged in Africa at least 500 years ago (Engel *et al.* 2016).

Currently, the virulence of the specific USUV lineages is unknown, and further studies are necessary to determine the biological characteristics of each lineage.

When comparing USUV to other JEV serocomplex viruses, the closest relative that can be found is MVEV, which exhibits 73% and 82% identity at the nucleotide and amino acid levels, respectively. JEV and WNV exhibit 71% and 68% identity with USUV at the nucleotide level and 81% and 75% at amino acid level, respectively (Bakonyi *et al.* 2004).

### 1.3 Cellular Tropism and Pathogenesis of USUV

USUV can infect cells of various tissues types derived from humans and a wide variety of animal species (Bakonyi *et al.* 2005).

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USUV infection on birds is often characterized by encephalitis, neuronal necrosis, hepatosplenomegaly, myocardial degeneration and necrosis of the liver and spleen (Chvala *et al.* 2004; Bakonyi *et al.* 2004; Calzolari *et al.* 2012; Weissenböck *et al.* 2003). However the demyelination of infected neurons was found to be a unique feature of USUV infection (Weissenböck *et al.* 2004).

Upon infection, human can develop a variety of signs such as encephalitis, meningitis and fever. USUV is not considered a significant human pathogen but it is rarely pathogenic in immune-compromised patients (Cavrini *et al.* 2009; Pecorari *et al.* 2009). These organ lesions can be regarded as a cause of death, because they were generally associated with sites of viral replication. However, not all of the mentioned lesions are always existing together.

USUV does not have a specific cell, tissue or organ tropism but, on the contrary, it is present in a large variety of cells (neurons, different epithelial cells and mesenchymal cells). The cause of death in birds is most likely due to a multi-organ failure, and brain lesions seem to play a central role (Chvala *et al.* 2002).

After the uptake into the cell by endocytosis, the viral particles are transported to the endosome. Here, the acidic environment enables the release of the capsid into the cytoplasm. After replication of the genome and synthesis of the viral proteins, the viral particles are assembled and the maturation takes place in the endoplasmic reticulum and are released by exocytosis.

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In relation to genus *Flavivirus*, autophagy is associated with multiple aspects of replication and pathogenic of some members of this genus, including DENV (Khakpoor *et al.* 2009); JEV (Li *et al.* 2012) and USUV (Blázquez AB *et al.* 2013).

Some viruses, including USUV, can take advantage of the autophagic process by incorporating the components of this cellular pathway in their own replication (Blázquez AB *et al.* 2013; Miller S and Krijnse-Locker J 2008).

“Autophagy” literally means “self-digestion” as it is a cellular process by which cytoplasmic components are isolated into double-membrane vesicles and degraded to maintain cellular homeostasis. In addition to this, “Autophagy” constitutes an evolutionarily ancient process of survival during different forms of cellular stress, including infection with viruses (Orvedhal A and Levine B 2008; Mizushima *et al.* 2008).

Upon infection, USUV induced the Xbp-1 pathway activation of the unfolded protein response, revealed by the splicing of Xbp-1 mRNA. It also increased cytoplasmic aggregation of microtubule-associated with the lipidated form of “protein 1 light chain 3” (LC3) (Weissenböck *et al.* 2004), which are considered as marker of autophagosome formation during viral infections that facilitates replication and immune evasion (Yu *et al.* 2006).

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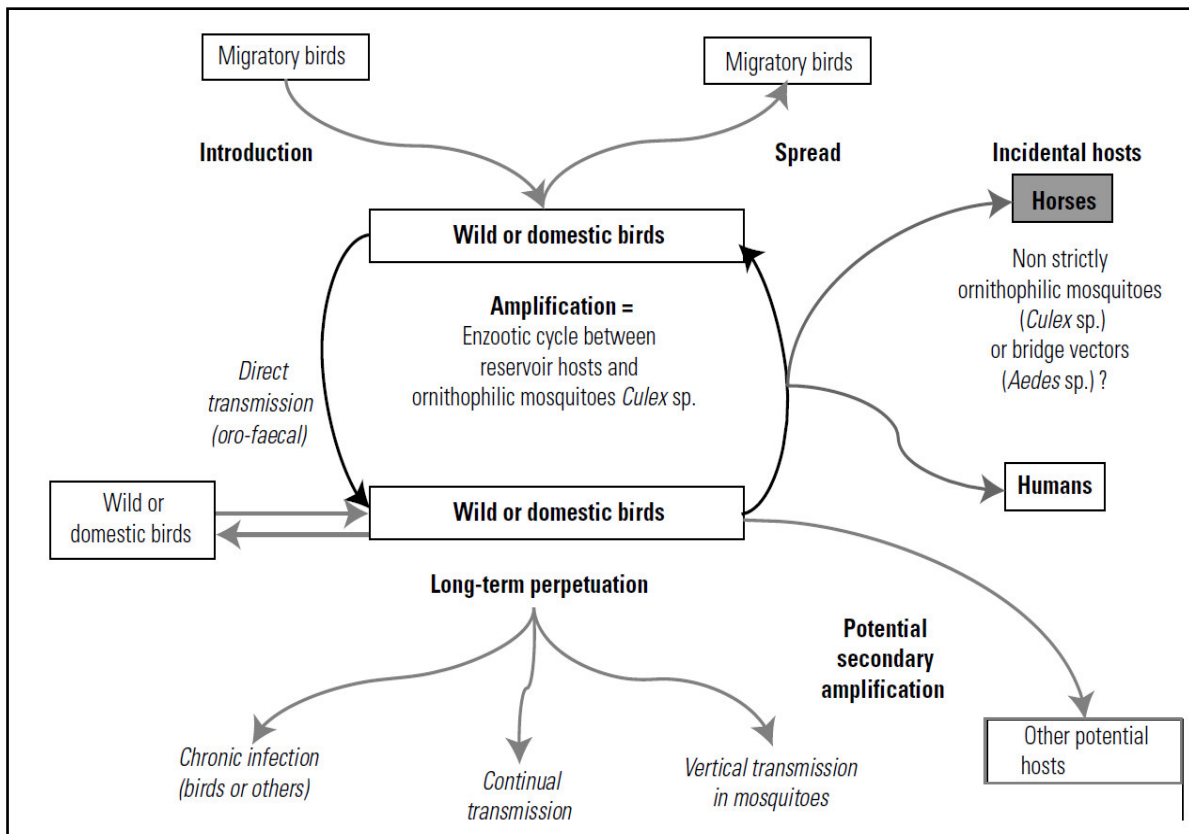
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### 1.4 Enzootic cycle, reservoir animals and “bridge-vectors”

The natural transmission cycle (**fig. 1.4**) occurs between ornithophilic mosquitoes (vector) and birds that serve as reservoir or amplifying host.



**Fig. 1.4** *Usutu* virus transmission cycle (from Pradier *et al* 2012)

Many mosquitoes and avian species (**table 1.3**) are involved in perpetuating the USUV life cycle (Vazquez A *et al.* 2011; Steinmetz HW *et al* 2011).

<i>Species</i>	<b>Common Name</b>	<b>Country (year)</b>
<i>Dendrocopos major</i>	Great spotted woodpecker	Belgium (2014)
<i>Pyrrhula pyrrhula</i>	Bullfinch	
<i>Columba livia domestica</i>	Domestic pigeon	Greece (2014)
<i>Turdus philomelos</i>	Song thrushes	Spain (2012)
<i>Turdus merula</i>	Eurasian blackbird	Italy (2010–2011) Germany (2011) Hungary (2003–2006) Austria (2001–2005)
<i>Alcedo atthis</i>	Common kingfisher	Germany (2011)
<i>Serinus canaria domestica</i>	Canary	
<i>Alectoris rufa</i>	Partridge	Italy (2010–2011)
<i>Asio otus</i>	Long-eared owl	
<i>Caprimulgus europaeus</i>	Nightjar	
<i>Garrulus glandarius</i>	Eurasian jay	
<i>Larus michahellis</i>	Yellow-legged gull	
<i>Pica pica</i>	Eurasian magpie	
<i>Streptopelia decaocto</i>	Eurasian collared dove	
<i>Ardea cinerea</i>	Grey heron	Germany (2011)
<i>Merops apiaster</i>	Eurasian bee-eater	Italy (2010–2011)
<i>Passer domesticus</i>	House sparrow	
<i>Picus viridis</i>	Eurasian green woodpecker	
<i>Sturnus vulgaris</i>	Common starling	
<i>Strix nebulosa</i>	Great grey owl	Germany (2011) Austria (2001–2002)
<i>Gallus gallus domesticus</i>	Chicken	Italy (2007–2009) Switzerland (2006–2007) England (2006)
<i>Spheniscus humboldti</i>	Humboldt penguin	Switzerland (2006–2007)
<i>Phoenicopterus ruber</i>	Greater flamingo	
<i>Dacelo novaeguineae</i>	Laughing kookaburra	
<i>Ciconia ciconia</i>	White stork	Austria (2006–2007)
<i>Leptoptilos crumeriferus</i>	Marabou stork	
<i>Neophron percnopterus</i>	Egyptian vulture	
<i>Bubo bubo</i>	Eurasian eagle owl	
<i>Bubo scandiacus</i>	Snowy owl	
<i>Strix uralensis</i>	Ural owl	

**Table 1.3** Avian species infected with USUV in Europe (from Ashraf *et al.* 2015)

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Mosquitoes are not strictly ornithophilic, in particular the genus *Culex*, are called “bridge-vector” because facilitate viral transmission to incidental host like humans, horses and rodents. Humans and horses are considered dead-end hosts due to their inability to develop a sufficient viremia to infect mosquitoes (Kramer *et al.* 2008; Weaver *et al.* 2010).

In 2015 in Germany USUV was also isolated from bats for the first time (Cadar *et al.* 2014) proving that it is able to emerge in other hosts. This observation raised questions regarding the USUV host range and the ability to adapt to new hosts.

### 1.5 Epidemiology of USUV

Following its discovery in South Africa in 1959, USUV was found in other African countries such as CAR (Central Africa Republic) (1969; 1981); Senegal (1974; 1993; 2007) (Adam F and Digoutte J-P 2014; Cornet M *et al.* 1979; Nikolay B *et al.* 2011; Chevalier *et al.* 2009) and Tunisia (2014) (Ben Hassine *et al.* 2014). USUV was typically isolated from mosquitoes, except for the strain “CAR 1981” which was isolated from a man who showed fever and rash (Institut Pasteur de Dakar 1984). The virus then moved from Africa into Europe, presumably carried by migratory birds and their related mosquitoes.

The first emergence of USUV in Europe was reported in Austria in 2001 and it derived from an episode of mass mortality in Eurasian blackbirds (*Turdus merula*) (Weissenböck *et al.* 2002).

Interestingly, a similar death of blackbirds was also reported in Italy in 1996 (Mani *et al.* 1998) but the causes, in this instance, remained unknown. Only in 2013, the partial nucleotide sequence of that unknown virus was compared with the Austrian strain “Vienna

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2001”, revealing a complete identity sequence (Weissenböck *et al.* 2013). On the base of this data, the emergence of USUV in Europe happened much earlier than previously thought.

In the following years, USUV was found in several other European countries (**fig. 1.5**) with virus isolation from mosquitoes, birds and bats. These countries included Hungary (2003-2006) (Bakonyi *et al.* 2007); Switzerland (2006) (Steinmetz HW *et al.* 2011); Spain (2006-2009) (Vázquez *et al.* 2011; Busquets *et al.* 2008); Italy (2006) (Calzolari *et al.* 2009; Manarolla *et al.* 2010; Savini *et al.* 2011; Tamba *et al.* 2011); Germany (2013) (Cadar *et al.* 2014) and Belgium (2014) (Garigliany *et al.* 2014)

USUV was detected in Sardinia for the first time in 2013 from mosquito’s pool (*C. pipiens*).

Moreover, USUV infection has also been proved serologically in birds in England (2001-2004) (Buckley *et al.* 2006), Czech Republic (2005) (Hubálek *et al.* 2008), Spain (2003–2006) (Figuerola *et al.* 2007), Poland (2006) (Hubálek *et al.* 2008), Switzerland (2006) (Steinmetz HW *et al.* 2007), Germany (2007) (Linke *et al.* 2007), Italy (2007) (Lelli *et al.* 2008), and Greece (2014) (Chaintoutis *et al.* 2014). The recurrence of the virus in Italy (2010–2011) (Calzolari *et al.* 2012; Calzolari *et al.* 2013), Germany (2011) (Becker *et al.* 2012), Spain (2012) (Höfle *et al.* 2013), and Czech Republic (2011–2012) (Hubálek *et al.* 2014) suggests persistence of the transmission cycle in the affected areas, possibly through overwintering mosquitoes (Vázquez *et al.* 2011).

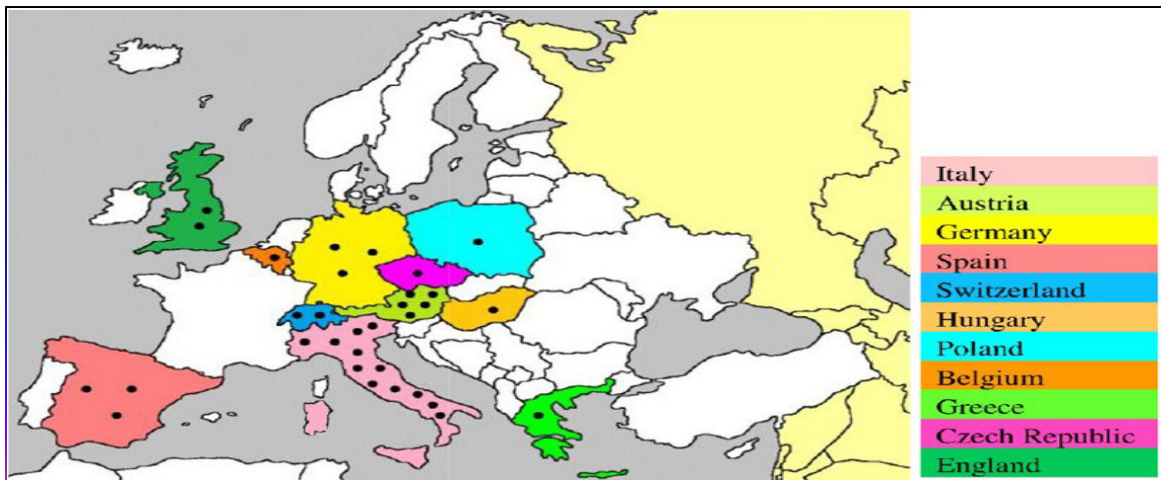
In the summer 2016, the virus was detected for the first time in the continent of South America, specifically in Colombia (Paniz-Mondolfi AE *et al.* 2016).

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**Fig. 1.5** Geographic locations of USUV-related epidemiological studies on birds and mosquitoes in Europe (from Ashraf *et al.* 2015).

Following its emergence in Europe, USUV has proven to be highly pathogenic for several species of wild birds, especially Eurasian blackbirds (*Turdus merula*) (Bakonji *et al.* 2004).

In addition to avian species, USUV has also been detected in humans. The first reported case of USUV infection was observed in Africa in two patients with fever, rash and jaundice (Nikolay *et al.* 2011).

Subsequently, in Europe two USUV-positive cases of meningoencephalitis were reported in immune-compromised patients in Italy (Cavrini *et al.* 2009; Pecorari *et al.* 2009; Gaibani *et al.* 2010). The first case was developed following orthotropic liver transplantation with an infected organ donor, and the second case was found in a cancer patient.

Recently, the first 5 cases of USUV specific-IgG positive sera in healthy blood donors have been reported, confirming the theory that USUV actively circulates amongst asymptomatic subjects in Europe (Gaibani *et al.* 2012; Allering *et al.* 2012).

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Therefore, the introduction onto a new continent of a previously exotic flavivirus that is closely related to other pathogenic flavivirus is a potential serious threat for a wide range of avian and mammalian species, including human (Bakonyi *et al.* 2004).

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## Chapter 2

### Aim of the Project

This research has been carried out by the Veterinary Medicine Department, Infectious Diseases Division of the University of Sassari, and the *Istituto Zooprofilattico Sperimentale della Sardegna* “G. Pegreff”, Animal Health Department, Virology and Entomology Division.

The aim of the project is the study of USUV circulating in Sardinia through the screening of surveillance samples.

On the base of positive samples, we have then proceeded to full genomic sequencing through the technique of “Primer-Walking” with the use of primers which have been designed by us.

From the data acquired, we have then proceeded to molecular characterization and phylogenetic analysis of strains that have been found, with the aim of investigating mutations associated with increased virulence or associated with geography - or host-adaptation.

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## Chapter 3

### Materials and Methods

#### 3.1 Entomological surveillance

The aim of entomological surveillance is to identify the mosquitoes involved in viral transmission and their seasonal abundance in the framework of the entomological activities, as defined in the Italian surveillance plan for WNV. In Sardinia we are also able to expand this surveillance plan for USUV.

In recent years, there have been increasing global reports of diseases due to *Arboviruses* (arthropod-borne viruses) (Gratz NG, 2006; Weaver SC and Reisen WK, 2010; Hubàlek Z, 2008). The detection of viruses in Invertebrate vectors and Vertebrate reservoir hosts is an important part of public health surveillance systems that allows the analysis of the intensity and seasonality of viral circulation in the environment. It provides data for the timely planning of preventive measures such as blood donation screening (CDC Guidelines for Surveillance, Prevention, & Control. 3<sup>rd</sup> revision, 2011).

Surveillance based on mosquitoes, wild and synanthropic birds sampling can provide early detection of the virus circulation before the onset of the disease in humans and horses (Calzolari *et al.* 2015).

Entomological surveillance in Sardinia was conducted using CDC light-traps operating for 24 hours in 35 geo-referenced sites every two weeks, from April until October 2015 or until two consecutive catches were detected without finding mosquitoes.

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Mosquitoes were identified to specie level by using morphological characteristics according to four classification keys (Stojanovich CJ and Scott HG, 1997; Schaffner *et al.* 2001; Severini *et al.* 2001; Becker *et al.* 2010).

Mosquitoes were pooled according to species, sex, place and date of sampling and on whether they were engorged or not, with a maximum of 50 individuals per pool.

To avoid cross-contamination due to possible loss of mosquito parts, the pools were obtained by handling specimens individually with tweezers.

The batches of mosquitoes were stored in 2 ml polypropylene cryotubes (Eppendorf) and frozen at -80 °C, up to their biomolecular analysis.

One tungsten ball (Tungsten Carbide Beads, Qiagen) of 3 mm in diameter, was added to each 2 ml tube. Different amount of PBS 1X were added to each tube according to the number of stored mosquitoes (350 ul up to 10 specimens; 500 ul up to 30 specimens; 600 ul up to 50 specimens).

The samples were then ground for 1 min and 30 sec (frequency 17 1/s) using the homogenizer TissueLyserII (Qiagen) and centrifuged in a refrigerated centrifuge at 10,000g for 20 minutes at 4 °C. Finally, samples were submitted to bio-molecular analysis through specific Real Time RT-PCR for the detection of USUV (Cavrini *et al.* 2011).

### **3.2 Birds surveillance**

A total of 525 birds were collected within the specific wildlife population control program in 2015 and were checked for the presence of USUV.

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The active surveillance was carried out on synanthropic birds. Weekly samples of approximately 20 - 40 birds that were caught or shot were collected. Monitoring mainly focused on the European magpie (*Pica pica*); Eurasian jay (*Garrulus glandarius*) and in particular on the Hooded crow (*Corvus cornix*).

The corvid is considered as a pest, due to its wide diffusion and eating habitat.

The passive surveillance was carried out on animals found dead in the field or deceased in wildlife rehabilitation centers.

Birds were necropsied and for each specimen we took the brain and a pool consisting of spleen, heart and kidney separately.

Homogenate was made to 10% in PBS (0.5 g sample in 5 ml PBS 1X). Samples were then ground using the homogenizer Omni Bead Ruptor 24 (Omni International, Inc., USA) with the following program: two steps to 45 sec with a pause for 15 sec; frequency 5,65 1/s. Twenty (2.8 mm in diameter) Ceramic Beads Bulk (Omni International, Inc., USA) was added to each 5 ml tube.

Viral RNA was extracted from 100 ul homogenate sample (pool of mosquitoes; organs) using the commercial kit Biosprint 96 One-for-all Vet Kit (384) (Qiagen) according to the manufacturer's instructions.

After RNA extraction with the automatic nucleic acid extractor Biosprint 96 (Qiagen) we submitted them to biomolecular analysis; samples from every bird were processed individually. All the samples were assessed with a specific Real-time RT-PCR for the detection of USUV RNA (Cavrini *et al.* 2011).

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### 3.3 Phylogenetic analysis

For phylogenetic analysis, the most informative genes of USUV, and generally for *Flavivirus* reported in bibliography, are the gene *E*, which encodes Envelope protein, and the gene *NS5*, which encodes RNA dependent RNA polymerase.

Initially, a preliminary amplification of partial *NS5* and *E* gene region was made before sequencing the entire genome of our isolates of USUV.

The purpose of this step was not only related to phylogenetic analysis but also to obtain information about the origin of our isolates (example European or Africans lineages), in order to develop specific primers for the whole genome sequencing and to have a strong confirmation of USUV positivity in our isolate based on data sequence.

Firstly, we tried to use the degenerated primers specific for *Flavivirus* designed by Sanchez-Seco MP *et al.* 2005 and Kuno *et al.* 1998 but unexpectedly we did not have any amplification on our samples.

After these negative results, we designed new primers to cover the partial genomic regions of the genes *E* and *NS5*. Primers are developed on viral variant obtained from a positive sample.

The pairs of primer designed by us were:

gene *NS5*:

Usuv8885 F TAAGAGGAAGGTCAACAGCA /

Usuv9597 R TTCCTGCCCAATCACTCCTT (712 bp)

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gene *E*:

Usuv1124 F CACGAACCTGGCTGAAGTGA /

Usuv1789 R CTGGAAYAGCTCCTGCCAAK (665 bp)

Thermo cycler parameters (except T° annealing 60°C) and PCR mix reaction are the same to the primers used for the full-length genome amplification.

We analysed all the isolates found but surprisingly we obtained only the partial sequence of *NS5* and *E* genes of two isolates, 91418 (2013) and 62059/4 (2015).

After sequencing, they confirmed the USUV positivity and a very close relation with Italian strains “Bologna2009” and “Italia2009”.

Based on these results, however, we proceeded into analysing only the isolates 91418 (2013) and 62059/4 (2015).

With data analysis, the gene *NS5* has proved globally much more informative in comparison to the gene *E*, and for this reason we decided to use the gene *NS5* for the phylogenetic analysis of USUV.

List USUV strains used for phylogenetic analysis:

1. Usutu virus strain UR-10-Tm polyprotein gene, complete cds [KX555624.1](#)
2. Usutu virus strain OS1-10-Tm polyprotein gene, complete cds [KX555628.1](#)
3. Usutu virus strain SE1-10-Tm polyprotein gene, complete cds [KX555626.1](#)
4. Usutu virus strain Bologna 2009, complete genome [HM569263.1](#)
5. Usutu virus strain Italia 2009, complete genome [JF266698.1](#)

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6. Usutu virus strain Vienna 2001 from Austria, complete genome [AY453411.1](#)
7. Usutu virus strain Meise H polyprotein gene, complete cds [JQ219843.1](#)
8. Usutu virus isolate Budapest, complete genome [EF206350.1](#)
9. Usutu virus strain V86, complete genome [KJ438781.1](#)
10. Usutu virus strain V20, complete genome [KJ438769.1](#)
11. Usutu virus strain V191, complete genome [KJ438779.1](#)
12. Usutu virus isolate S.nebulosa-7890/Fra/2016, complete genome [KY128481.1](#)
13. Usutu virus strain V248, complete genome [KJ438774.1](#)
14. Usutu virus strain V321, complete genome [KJ438766.1](#)
15. Usutu virus strain 3358, complete genome [KJ438721.1](#)
16. Usutu virus gene for polyprotein, genomic RNA, isolate BH65/11-02-03 [HE599647.1](#)
17. Usutu virus strain VB-15-Tm polyprotein gene, complete cds [KX555629.1](#)
18. Usutu virus strain V211, complete genome [KJ438764.1](#)
19. Usutu virus strain V72, complete genome [KJ438761.1](#)
20. Usutu virus strain V49, complete genome [KJ438756.1](#)
21. Usutu virus strain V210, complete genome [KJ438763.1](#)
22. Usutu virus strain V10, complete genome [KJ438760.1](#)
23. Usutu virus strain V245, complete genome [KJ438747.1](#)
24. Usutu virus strain V93, complete genome [KJ438746.1](#)
25. Usutu virus strain 6424, complete genome [KJ438736.1](#)
26. Usutu virus strain 1477, complete genome [KJ438705.1](#)
27. Usutu virus isolate BAT2USUTU-BNI, complete genome [KJ859683.1](#)
28. Usutu virus isolate BAT1USUTU-BNI, complete genome [KJ859682.1](#)
29. Usutu virus isolate HB81P08, complete genome [KC754955.1](#)
30. Usutu virus isolate ArD101291, complete genome [KC754956.1](#)
31. Usutu virus isolate ArD192495, complete genome [KC754957.1](#)
32. Usutu virus strain Bonn, complete genome [KM659877.1](#)
33. Usutu virus isolate S.nebulosa-6004/NL/2016, complete genome [KY128482.1](#)
34. Usutu virus isolate ArD19848, complete genome [KC754954.1](#)
35. Usutu virus strain SAAR-1776 from South Africa, complete genome [AY453412.1](#)
36. Usutu virus strain Spain MB119/06, complete genome [KF573410.1](#)

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As outgroup we included the *Murray Valley* encephalitis virus (MVEV), by using the reference strain “MVEV\_NC000943.1”.

Pairwise/multiple sequence alignments and sequences similarities were calculated using the ClustalX software (Larkin *et al.* 2007).

The evolutionary history of USUV was inferred using the Neighbor-Joining method (NJ) (Saitou and Nei 1987) identified as the best-suited evolutionary model for our data. The phylogenetic tree was obtained by applying Neighbor-Joining (NJ) algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) method (Tamura *et al.* 2004) and were calculated as units of the number of base substitutions per site. A discrete Gamma distribution was used to model evolutionary rate differences among sites (parameter = 0.45880082). The analysis involved 39 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. Positions containing gaps and missing data were disposed of.

A total of 1490 positions were included in the final dataset and evolutionary analyses were conducted in MEGA6 (Tamura *et al.* 2013). Statistical support for internal branches (shown next to the branches) of the trees was evaluated by bootstrapping with 100 iterations (Felsenstein 1985). NJ (Saitou and Nei 1987) trees and consensus values were generated using the same software.

### 3.4 Whole genome sequencing Protocol

Regarding the molecular characterization of USUV, it is very important to know the complete genome sequence, because there are many hot points for the virulence spread in the viral genome.

However, initially we had to solve a problem. In fact, in the current method of sequencing the whole genome of USUV with “Primer-Walking”, we had too many PCR reactions, and this was not suitable especially with a small amount of RNA sample to work with, like in our case.

To solve our problem we developed a new protocol divided in 4 steps:

- 1) cDNA optimization
- 2) new primers design to cover the whole genome in a few PCR reactions
- 3) PCR products cloning
- 4) Sequencing

#### 1. cDNA optimization

The first step we took was the cDNA optimization. At first we made two different types of cDNA using two different specific primers for USUV: UsuvF1 and Usuv11066Rdeg. This step was undertaken to ensure the retro-transcription of the entire viral genome.

Then, we tested several combinations of primer concentration and different amount of sample by using a specific Real time RT-PCR for USUV (Cavrini *et al.* 2011). This was

done to try to limit the amount of RNA sample. The best arrangement was 20 uM primer concentration and 2,5 ul of RNA sample.

### cDNA Protocol

We used the commercial Kit “SuperscriptII reverse transcriptase”, (Invitrogen).

It is divided in 3 steps:

1<sup>st</sup> Step Program name: “**65 Usutu**”

Add:

- RNA 2.5 ul
- Primer 1 ul
- BSA 1X 0.5 ul
- dNTPs 10 mM 1 ul
- H<sub>2</sub>O milliQ 4.5 ul

for 5' at 65 °C ;

2<sup>nd</sup> Step Program name: “**Mix Usutu**”

Add:

- 5X first-strand buffer 4 ul
- DTT 0,1 M 2 ul
- RNasi OUT 40U/ul 1 ul

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Mix gently pipetting up and down, then:

for 2' at 42 °C ;

3<sup>rd</sup> Step Program name: “**cDNA Usutu**”

Add:

- 1 ul *reverse transcriptase* 200 U/ul

Mix gently pipetting up and down, then:

for 50' at 42 °C → for 15' at 70 °C ;

## 2. New primers design to cover the whole genome in a few PCR reactions

We designed new primers that overlapped between them to cover the whole genome in a few PCR reactions. Primers are developed on viral variant obtained from a positive sample.

To design our primers we aligned several strains of USUV from African and European origin using MUSCLE software (available at <http://www.ebi.ac.uk/Tools/msa/muscle/>). (See Table **S1** in the supplemental material).

List USUV strains used to design our primers:

Spain\_MB119/06\_2006 (KF573410)

Dakar\_ArD192495 (KC754957)

Senegal\_ArD101291\_1993 (KC754956)

Germania\_V45\_2011 (KJ438770)

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Germany_V382_2013	(KJ438758)
Vienna_2001	(AY453411)
Bologna_2009	(HM569263)
Italia_2009	(JF266698)
Dakar_ArD19848_1974	(KC754954)
South_Africa_SAAR-1776	(AY453412)

Primer design performed using the free software available on:

[www.promix.cribi.unipd.it/cgi-bin/promix/melting](http://www.promix.cribi.unipd.it/cgi-bin/promix/melting)

PCR reaction Protocol (50 ul)

We used the commercial Kit “Platinum *Taq* DNA Polymerase” (Invitrogen)

PCR mix:

10X PCR Buffer, -Mg	5 ul
50 mM MgCl <sub>2</sub>	1.5 ul
10 uM dNTP mix	1 ul
10 uM forward primer	1 ul
10 uM reverse primer	1 ul
Template cDNA	4 ul

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Platinum *Taq* DNA Polymerase 2 U/ rxn      0.2 ul  
 H<sub>2</sub>O milliQ, nuclease free                      36.3 ul

Thermo cycler parameter:

Initial denaturation 2' at 94°C

30sec at 94°C

30sec at (depending on primers T° melting) } x 35

1' / Kb (depending on the length of amplified)

Infinite hold at 4°C

T° annealing:

- PCR reactions: **2; 3; 5** (using the cDNA made with the primer UsuvF1) and **8** (using the cDNA made with the primer Usuv11066Rdeg) is 60°C ;
- PCR reactions: **4** and **6** is 55 °C (using the cDNA made with the primer UsuvF1).
- PCR reaction **1** is 62 °C (using the cDNA made with the primer Usuv11066Rdeg).
- PCR reactions: **7; 9, 10, 11** and **12** is 58°C (using the cDNA made with the primer Usuv11066Rdeg).

Pairs of primer used for the full-length amplification of USUV genome:

1) UsuvF1deg AGWYGTTBGYCTGYGTGAGC / Usuv327R TGCCGTGGTCTTGTTGATGC (327 bp)

2) Usuv232F TTTGTGCTGGCCTTGATGAC / Usuv1246R CGTACGTGTCCTCAGCTCTC (934 bp)

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- 3) Usv1124F CACGAACCTGGCTGAAGTGA / Usv2353R TGATCCAGGACATGCCACCG (1229 bp)
- 4) Usv2295F GTACATCAGGTCTTTGGAGG / Usv4693R TGTAACGGCTCATGATACGG (2398 bp)
- 5) Usv4578F TGGCTCACTGTCAAGTACGC / Usv5509R CAATGTAGCCTCGTGCTGCTAT (931 bp)
- 6) Usv5443F ACCTCTTTGTGATGGATGAG / Usv6935R GTTCTCTCTAACATTCCGTA (1492 bp)
- 7) Usv6861F GCTGTGTTTCTGATCTGTGT / Usv8956R TGGCACTGCTCCATTGGTTC (2095 bp)
- 8) Usv8885F TAAGAGGAAGGTCAACAGCA / Usv9994R ACAACAGGAGCCACATCTGA (1109 bp)
- 9) Usv9643F\* GAGAACGGAGAAGAAAGGGT / Usv10823R\* ACCAGTTCGCATCACCGTCT (1181bp)
- 10) UsvADF\* (10646) GAAAGCCCCTCAGAACCGTTTC /  
Usv11066Rdeg AGATCCTGTGGTCTWGYCC (420 bp) or
- 11) UsvADF\* (10646) GAAAGCCCCTCAGAACCGTTTC /  
Usv11014R\* AGATCCTGTGKTCTWSYYCMCCAYCAG (420 bp) or
- 12) Usv10794F\*\* CAAGCGAACAGACGGTGATG /  
Usv11027R\* GCGCTCTGTGCCTTGTGGTTGAT (233 bp)

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Pairs of primer used for closing gap (because the amplicon was too long to be sequenced in one-step by Sanger's method) in reactions 4 and 7:

- 1) Usuv6901F GTGGCTGCCAATGAATACGGAA / Usuv7845R TGGGTGTCCTCCAGTTTTGT (944bp)
- 2) Usuv7769F AGAGGCCATCACTGAAGTCG / Usuv8908R CGTTGCTGTTGACCTTCCTC (1139bp)
- 3) Usuv3167F TTGGAGTGATGGCGTTGTTG / Usuv4600R TTGCGTACTTGACAGTGAGC (1433bp)

(for these pairs primer the T° annealing is 60°C)

(\*) from (Cadaru *et al.* 2014)

(\*\*) from (Bakonyi *et al.* 2004)

Nucleotide position are according to the “Vienna2001” reference strain USUV genome sequence (AY453411) (Bakonyi *et al.* 2004). (See Table S2 in the supplemental material).

Therefore, with this protocol the whole genome of USUV could be sequenced from only 10 PCR reactions, which were far less in comparison to others protocols available in bibliography (Cadaru *et al.* 2014; Bakonyi *et al.* 2004).

Subsequently, the PCR products were all gel purified and cloned. Gel purification electrophoresis was carried out in a 1% Tris acetate-EDTA-agarose gel at 80 V for 60'.

The gel was stained with Gel Star<sup>TM</sup> Nucleic Acid Gel Stain, 10.000X concentrate in DMSO (LONZA Rockland, ME USA).

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Then, the band with the molecular size expected, was completely removed from the gel and purified with the commercial Kit Zymoclean Gel DNA Recovery Kit (200 Preps) (ZYMO RESEARCH, USA) according to the instructions of the manufacturer.

After gel purification, the PCR products were all cloned using the commercial kit “TOPO TA Cloning Kit for sequencing” (Invitrogen) that included “One Shot TOP10 Chemically Competent *Escherichia coli* cells” (Invitrogen) and the plasmid vector “pCR-4-TOPO”, according to the instructions of the manufacturer.

Plasmid DNA was extracted from positive colonies with “PureLink Quick Plasmid MiniPrep Kit” (Invitrogen) according to the instructions of the manufacturer.

To check if the correct insert was present, we made a specific enzymatic cleavage (EcoRI enzyme) on the plasmid with the following protocol:

Mix (20 ul)

EcoRI enzyme 0.3 ul

10x Buffer 3 ul

100x BSA 0.3 ul

H<sub>2</sub>O milliQ (depending on the sample concentration)

Plasmid DNA (depending on the sample concentration\*)

\*For each reaction needs average 600 ng of plasmid DNA;

Incubation was performed at 37°C for 2 hours. Lastly, we checked the band expected through a gel electrophoresis carried out in a 1% Tris acetate-EDTA-agarose gel at 80 V for 60 min.

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## Chapter 4

### Results

#### 4.1 Surveillance plan on birds and mosquitoes

In relation to the surveillance plan, during the year 2015, 5,345 adult mosquitoes representing 17 different species were collected (**table 4.1**). The most represented species were *O. caspius* with 1,454 specimen(27.2%); *C. pipiens* 994 (18.6%); *C. theileri* 764 (14.3%) and *O. detritus* 732 (13.7%).

SPECIES	TOTAL (%)
<i>Ochlerotatus caspius</i>	1454 (27.20)
<i>Culex pipiens</i>	994 (18.59)
<i>Culex theileri</i>	764 (14.29)
<i>Ochlerotatus detritus</i>	732 (13.69)
<i>Culiseta annulata</i>	580 (10.85)
<i>Culex spp.</i>	259 (4.84)
<i>Anopheles labranchiae</i>	171 (3.19)
<i>Anopheles algeriensis</i>	155 (2.89)
<i>Aedes spp.</i>	86 (1.60)
<i>Culiseta longiareolata</i>	46 (0.86)
<i>Ochlerotatus mariae</i>	28 (0.52)
<i>Aedes vexans</i>	21 (0.39)
<i>Aedes albopictus</i>	20 (0.37)
<i>Culiseta subochrea</i>	9 (0.16)
<i>Culiseta spp.</i>	9 (0.16)
<i>Culex hortensis</i>	5 (0.09)
<i>Coquillettidia richiardii</i>	5 (0.09)
<i>Anopheles spp.</i>	4 (0.07)
<i>Aedes vittatus</i>	1 (0.01)
<i>Culex impudicus</i>	1 (0.01)
<i>Orthopodomyia pulchripalpis</i>	1 (0.01)

**Table 4.1** Species of mosquitoes caught during 2015 (from Rossi *et al.* 2016).

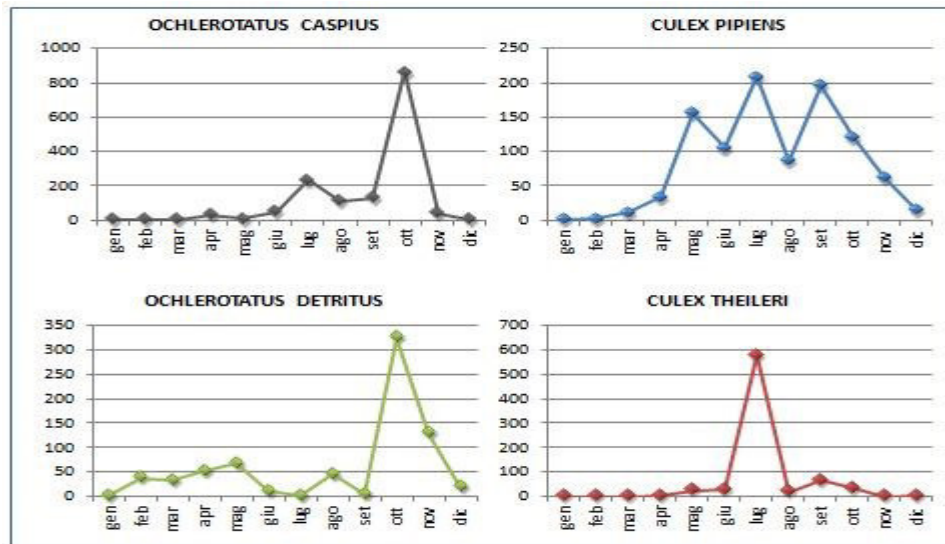
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The seasonal abundance (**table 4.2**) peak of *O. caspius* and *O. detritus* were recorded in October with a maximum of 859 and 324 individuals, respectively while *C. pipiens* and *C. theileri* showed a peak in July with 208 and 577 adults, respectively.



**Table 4.2** Seasonal trends spp. *Culex* and *Ochlerotatus* (from Rossi *et al.* 2016).

The *Ochlerotatus* taxon was considered as an *Aedes* sub-genus (Savage *et al.* 2004).

These results confirm the gradual adaptation of the mosquito belonging to *Aedes* genus to our climate and environment which could be a very dangerous future health threat because the *Aedes* mosquito is the main vector for *Zika* virus (ZIKV).

During the year 2015, we also observed the over-wintering of mosquitoes in Sardinia, probably due to a drought condition.

Overall, 1,004 pools were sorted and tested for USUV and it was detected in 4 pools consisting of not-engorged females of *C. pipiens* captured from August to October in Siniscola (ID 54860/1), Oschiri (ID 59265), Alghero (ID 62059/4) and Olbia (ID 71253/2) municipalities.

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During 2015, 525 birds were collected within the specific wildlife population control programs and USUV was detected in a pool (spleen, heart, and kidney) of owl (*Athene noctua*) in Olbia (ID 81088) municipality.

In total we found eight isolates of USUV in Sardinia (**table 4.3**): five in 2015 which have been already described above, two isolates of USUV found in 2014 in Badesi (ID 67279/1) and San Teodoro (ID 80158) municipalities and finally, the first isolate of USUV found in Sardinia in 2013 in Sassari (ID 91418) municipality.

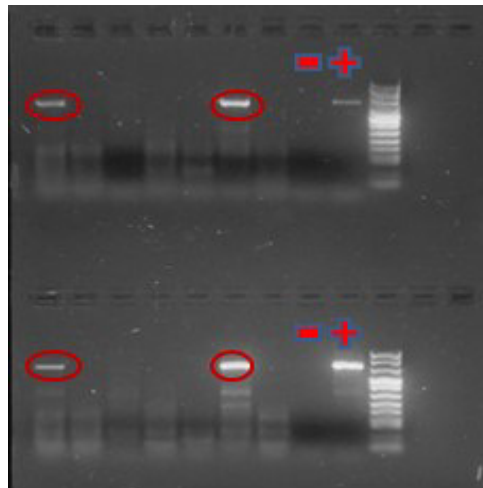
<b>ID sample</b>	<b>sample</b>	<b>year</b>	<b>sample screened 2013-15</b>
ID 91418	mosquito	2013	3124
<b>ID 67279/ 1</b>	<b>mosquito</b>	<b>2014</b>	<b>3124</b>
ID 80158	mosquito	2014	3124
<b>ID 54860/ 1</b>	<b>mosquito</b>	<b>2015</b>	<b>3124</b>
ID 59265	mosquito	2015	3124
<b>ID 62059/ 4</b>	<b>mosquito</b>	<b>2015</b>	<b>3124</b>
ID 71253/ 2	mosquito	2015	3124
<b>ID 81088</b>	<b>owl</b>	<b>2015</b>	<b>1835</b>

**Table 4.3** Positive samples for USUV found in Sardinia from 2013 to 2015.

All isolates were detected and confirmed by specific Real Time RT-PCR for USUV (Cavrini et al. 2011). However only the strains 91418 and 62059/4 were also confirmed with data sequence.

## 4.2 Phylogenetic analysis

We analyzed all the isolates found (**fig. 4.1**) but surprisingly we obtained only the partial sequence of *NS5* and *E* genes of two isolates, 91418 (2013) and 62059/4 (2015) and only by using the primers that were designed by us.



**Fig. 4.1** Photo gel electrophoresis for phylogenetic analysis; the top row in the gel represents the gene *E* while the row below represents the gene *NS5*. We used Marker8 (Roche).

Unfortunately, we did not achieve any result with the use of the primers developed by Sanchez-Seco MP *et al.* 2005 and Kuno *et al.* 1998.

Following these results, we proceeded only with the analysis of the isolates 91418 and 62059/4.

In order to see if they were different, we aligned the sequence of the two isolates with MUSCLE software (available at <http://www.ebi.ac.uk/Tools/msa/muscle/>) through preliminary data sequence, and the percentage of identity in both genes was of 100%. We gave to this type of sequence the temporary name of “USUTUSARD2013-2015”.

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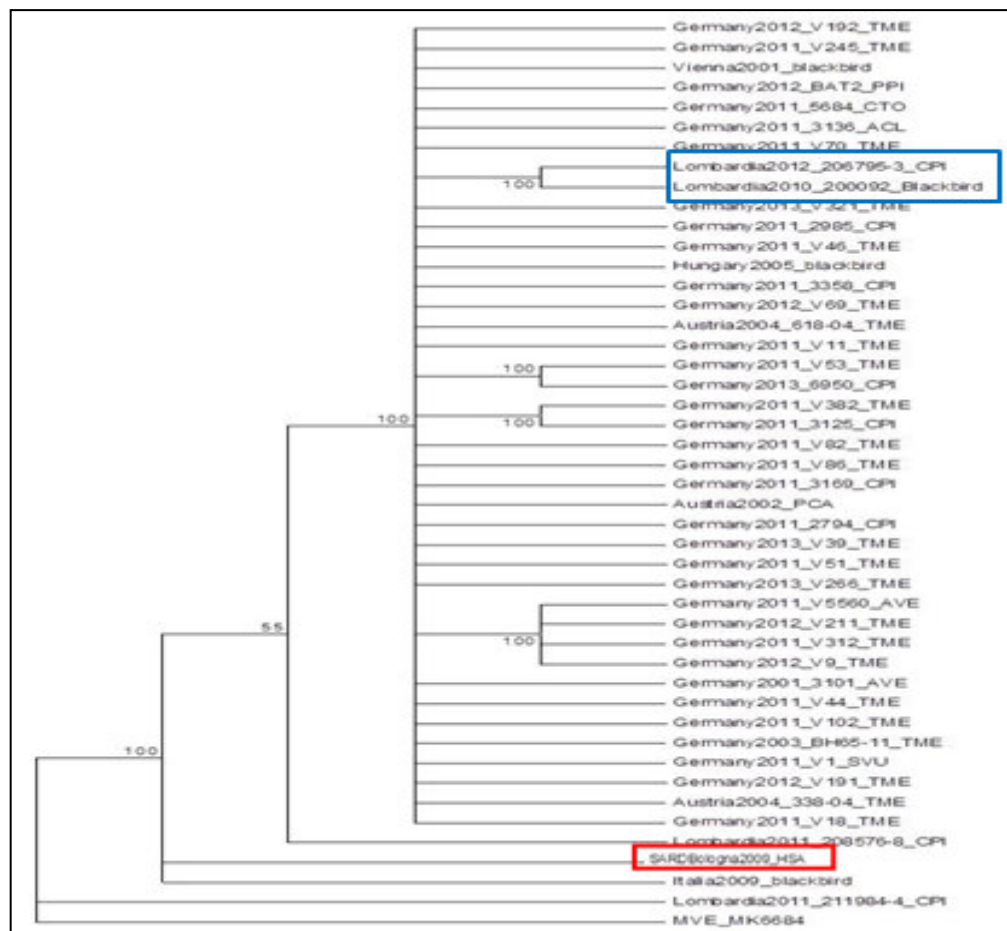
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Afterwards we compared these results with other sequences of USUV present in the database. The phylogenetic tree was obtained by MEGA software (Tamura *et al.* 2013) using the maximum parsimony in the analysis of taxa, simply because this is more suitable for much preserved sequences such as USUV genome.

In preliminary analysis, the gene *E* (fig. 4.2) did not prove to be more informative globally but it distinguished well between Italian strains.



**Fig. 4.2** Provisional phylogenetic tree of gene *E* USUV.

Instead, the gene *NS5* was much more informative globally, for this reason we decided to use it for the phylogenetic analysis of USUV. In addition, in order to differentiate better

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between the isolates 91418 and 62059/4 we decided to extend the sequence to investigate within the *NS5* gene from (8885-9597 nt) to (8885-10398 nt).

Percent Identity Matrix - created by Clustal2.1

1: 62059	100.00	99.46
2: 91418	99.46	100.00

In this way, we were able to discriminate between our strains and we designed them respectively in strain “**91418\_Sassari2013**” and strain “**62059\_Sardegna2015**”.

Subsequently, we compared them with all USUV strains available in the GenBank from African and European origin using MEGA6 software (Tamura *et al.* 2013) to be able to obtain the phylogenetic tree of *NS5* gene (**fig. 4.3**).

The phylogenetic tree shows that Sardinian strains are closely related to Italian strains, in particular with strain “Bologna2009”. This was an extremely interesting discovery because “Bologna2009” has been the first strain isolated from a human patient presenting with neurological symptoms (Gaibani *et al.* 2009).

However, Sardinian isolates show some unique molecular properties in comparison to all Italian strains, like the deletions in 3’-UTR which, until now have not been detected in Italy.

The phylogenetic analysis of the European strains demonstrated that they can be divided in 3 major groups defined by location of sampling: German group, Austrian group and Italian group.

On the other hand, Spanish strains are linked with African strains as well as strains “Holland2016” and “Bonn2014”. These strains probably belong to the third recent

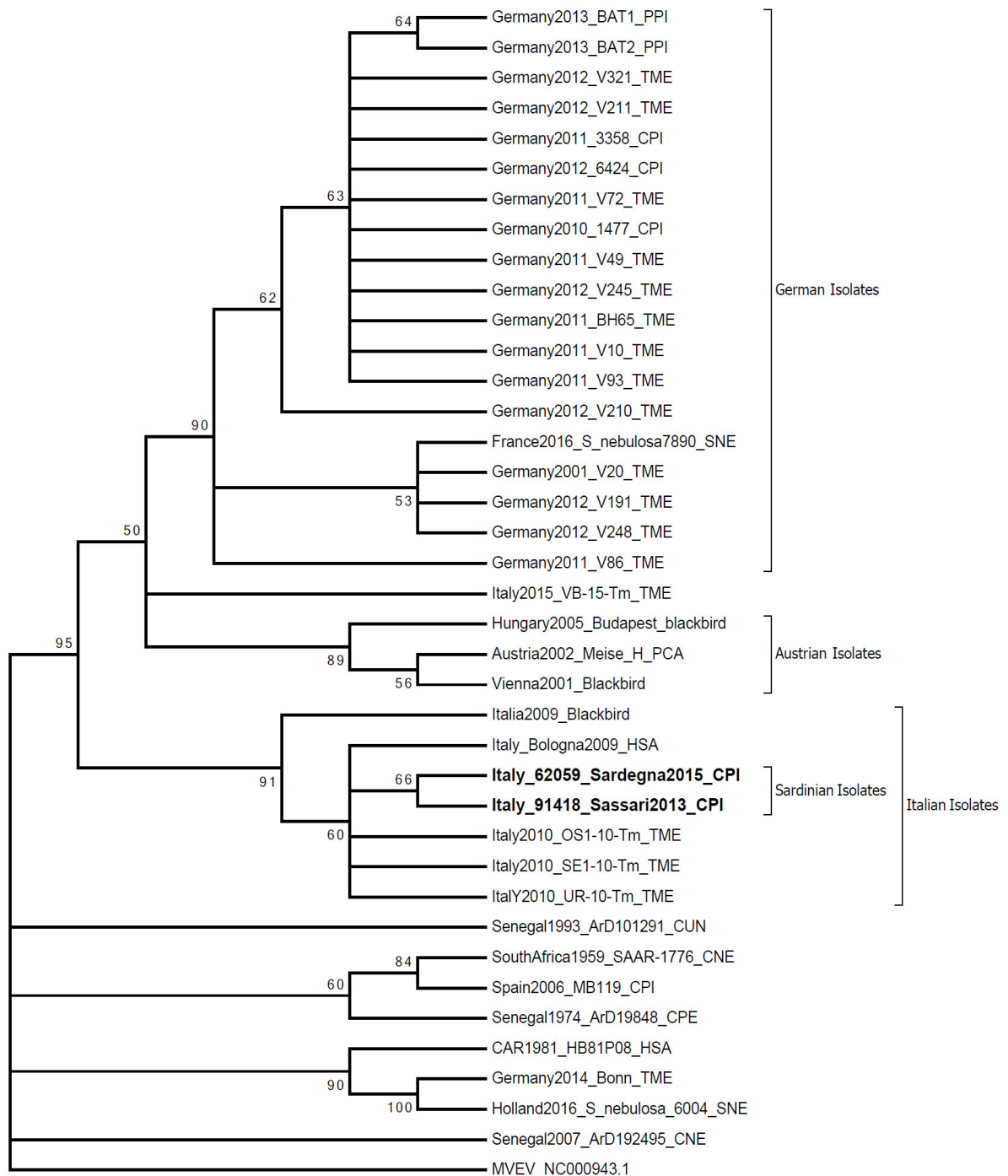
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independent introduction (Engel *et al.* 2016) in Western Europe (Spain) of USUV via migratory birds.



**Fig. 4.3** Evolutionary relationships among USUV strains based on *NS5* gene.

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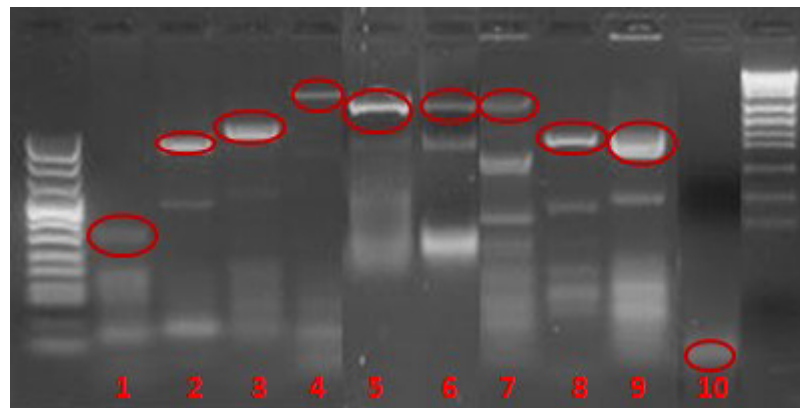
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### 4.3 Whole genome sequencing and molecular characterization

This protocol allows to sequence the whole genome of USUV with a smaller number of PCR reactions, specifically in only 10 PCR reactions, much less in comparison to others protocols available in bibliography (Cadar *et al.* 2014; Bakonyi *et al.* 2004).

The PCR reactions functioned well but there were a lot of unspecific band (**fig. 4.4**) because pool of mosquitoes are a very complex matrix. In fact, there are different type of “mosquito-only flavivirus” that maybe can cross-react between them. (Calzolari *et al.* 2012; Grisenti *et al.* 2015; Roiz *et al.* 2012; Vázquez *et al.* 2012).



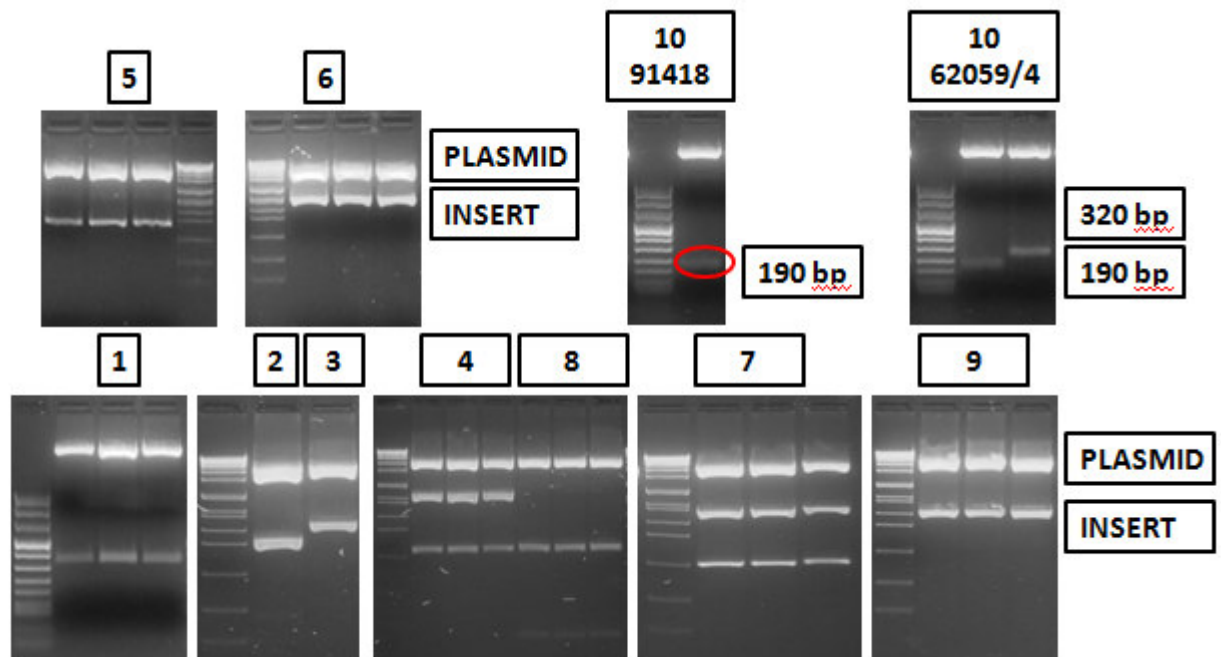
**Fig. 4.4** Gel electrophoresis for all PCR reactions to amplify the entire genome of USUV. Isolates 91418 and 62059/4 gave the same results except for the reaction 10.

We used Marker8 (Roche) on the left and Marker7 (Roche) on the right.

In relation to the 3'-terminal region, we had many difficulties in obtaining the expected amplification. For the isolate 91418 we obtained a band at 190 bp (but we expected a band at 420 bp) with the PCR reaction ADF/ 11066Rdeg. For the isolate 62059 we obtained two

bands at 190 bp and 320 bp (but we expected a band at 420 bp) with the PCR reaction ADF/11014R but after cloning and sequencing unfortunately they turned out to be unspecific amplification.

The PCR products have been all gel purified and cloned successfully (**fig.4.5**).

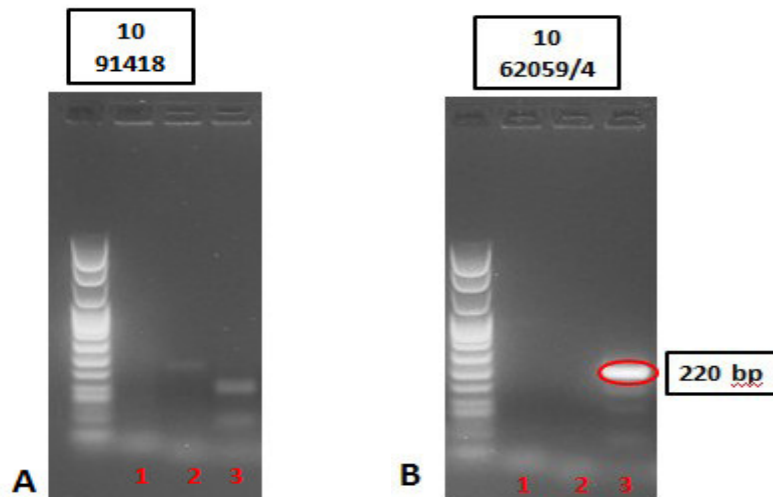


**Fig. 4.5** Gel electrophoresis to check if there were the correct inserts. Isolates 91418 and 62059/4 gave the same results except for the reaction 10. We used Marker8 and Marker7 (Roche).

Therefore, we tried again to use the previous primer combinations (pairs primer already described before) but the cDNA was made with "random hexamers" (Roche) rather than with a specific primer for USUV (11066Rdeg).

In addition, we tried to use a reverse primer (11027R) in a slight previous of the theoretical end of the viral genome.

We achieved amplification (**fig.4.6**) only with the pair primer 10746F/ 11027R (band expected at 220 bp) and only in the isolate 62059/4 (**B**). Unfortunately, we found no valid results for the isolate 91418 (**A**).



**Fig.4.6** The following pairs primer were used: (1) ADF/ 11066Rdeg; (2) ADF/11014R; (3) 10746F/ 11027R; We used Marker8 (Roche). After sequencing confirmed to be USUV.

This particular difficulty to amplify the 3'-terminal of viral genome can be explained by the fact that some USUV strains (in particular in the African lineages) have typically high variable size (short or large deletion). Therefore we assume that our isolates presented a long or short deletion.

BMR GENOMICS Company carried out the sequencing of plasmids and PCR products.

FinchTV software version 1.4.0 (GEOSPIZA, Inc.) (Available at [www.geospiza.com/finchty](http://www.geospiza.com/finchty)) analyzed and edited the chromatogram of each sequence.

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The nucleotide sequences were aligned using MUSCLE software (available at <http://www.ebi.ac.uk/Tools/msa/muscle/>) and confirmed by BLAST software (available at <https://blast.ncbi.nlm.nih.gov/Blast>).

The 91418 USUV isolate complete genome sequence was designated “**91418\_Sassari2013**” while the 62059/4 USUV isolate complete genome sequence was designated “**62059\_Sardegna2015**”.

After obtaining the complete genome sequences, we started the molecular characterization on our viral strains of USUV.

### **Isolate 91418\_Sassari2013**

The genome length for the isolate 91418\_Sassari2013 is of 10823 nt and contains one large open reading frame (ORF) comprising nucleotides (nt) 97 to 10401 that encodes a unique polyprotein precursor of 3424 amino acid (aa).

This strain is characterized for having a putative large deletion in the 3'-UTR.

Through a comparison of the entire genome nucleotide sequence between our isolates, the Percent Identity Matrix created by Clustal2.1 is:

```
1: 91418_Sassari2013 100.00 99.43
2: 62059_Sardegna2015 99.43 100.00
```

After this, we used BLAST software (<https://blast.ncbi.nlm.nih.gov/Blast>) to estimate the degree of identity among the strain 91418\_Sassari2013 and all the strains of USUV available in the GenBank at nucleotide level.

This analysis showed a Percent Identity Matrix of 99% with all USUV strains except African strains: "ArD101291" [KC754956.1](#) ; "HB81P08\_CAR\_1981" [KC754955.1](#) ; "ArD192495" [KC754957.1](#) ; "ArD19848" [KC754954.1](#) and "SAAR-1776\_South Africa" [AY453412.1](#) (98-97%) and the following European strains: "S.nebulosa-6004/NL/2016" [KY128482.1](#) (97%) and "Bonn" [KM659877.1](#) (97%).

This is closely related with the Italian strains of USUV, in particular with:

"UR-10-Tm\_2015" [KX555624.1](#) ; "Bologna2009" [HM569263.1](#) ; "SE1-10-Tm\_2010" [KX555626.1](#).

Usually the accumulation of mutations in *Arbovirus* is given by the transmission and infection modes as effect of alternation between the arthropod vector and avian or mammal hosts (Holmes EC 2003).

Regarding the UTRs, which is extremely important in *Flavivirus*, we analyzed the complete 5'-UTR sequence of this strain that is 96 nt long. Its sequence was closely related with all European strains of USUV and we have not found any unique mutation.

Comparing the complete 5'-UTR nucleotide sequence between our isolates, the Percent Identity Matrix created by Clustal2.1:

1: 91418_Sassari2013	100.00	96.88
2: 62059_Sardegna2015	96.88	100.00

We have not found any particular mutation in this genomic region.

The 3'-UTR sequence in USUV is typically high and variable in size. In the isolate 91418\_Sassari2013 is 425 nt long such as many African strains of USUV (**fig. 4.7**).

Spain2006_MB119_CPI	TGATGCGAACTGTTTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGAACTTAGGTTG
Holland2016_S_nebulosa_6004_SNE	TGATGCGACCTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGAACTTAGGTTG
Germany2014_Bonn_TME	TGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGAACTTAGGTTG
CAR1981_HB81P08_HSA	TGATGCG-----
Senegal1993_ArD101291_CUN	TGATGCGAACTG-TTCGTGGAAGGACTAG-----
Italy_Sardegna2015_CPI	TGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGAACTTAGGTTG
Germany2012_V211_TME	TGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGAACTTAGGTTG
Germany2012_V248_TME	TGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGAACTTAGGTTG
Germany2011_2794_CPI	TGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGAACTTAGGTTG
Germany2011_BH65_TME	TGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGAACTTAGGTTG
Germany2013_BAT1_PPI	TGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGAACTTAGGTTG
Germany2011_V10_TME	TGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGAACTTAGGTTG
Italy_Sassari2013_CPI	TGATGCGAACTG-TT-----
Vienna2001_Blackbird	TGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGAACTTAGGTTG
Hungary2005_Budapest_blackbird	TGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGAACTTAGGTTG
Austria2002_Meise_H_FCA	TGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGAACTTAGGTTG
Italy_Bologna2009_HSA	TGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGAACTTAGGTTG
Italia2009_Blackbird	TGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGAACTTAGGTTG
Italy2010_OS1-10-Tm_TME	TGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGAACTTAGGTTG

**Fig. 4.7** Alignment of several strains of USUV of 3'-UTR.

The 3'-UTR sequence of this strain was aligned with several sequences of USUV strains (strains list described below) available in the GenBank to find unique or specific mutations. We found a specific mutation in position 5 T→C related only with our strains (62059\_Sardegna2015) and the strains “Berlin2015” and “Spain\_MB119\_2006”.

List USUV strains used to investigate in the 3'-UTR:

1. Usutu virus isolate ArD101291, complete genome [KC754956.1](#)
2. Usutu virus isolate HB81P08, complete genome [KC754955.1](#)
3. Usutu virus strain Spain MB119/06, complete genome [KF573410.1](#)
4. Usutu virus clone Berlin2015\_ME/2015/ED-A, complete genome [KU664608.1](#)
5. Usutu virus isolate S.nebulosa-6004/NL/2016, complete genome [KY128482.1](#)
6. Usutu virus strain Bonn, complete genome [KM659877.1](#)
7. Usutu virus strain SAAR-1776 from South Africa, complete genome [AY453412.1](#)
8. Usutu virus strain V10, complete genome [KJ438760.1](#)
9. Usutu virus gene for polyprotein, genomic RNA, isolate BH65/11-02-03 [HE599647.1](#)
10. Usutu virus strain OS1-10-Tm polyprotein gene, complete cds [KX555628.1](#)
11. Usutu virus strain V211, complete genome [KJ438764.1](#)
12. Usutu virus strain V248, complete genome [KJ438774.1](#)

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13. Usutu virus isolate BAT1USUTU-BNI, complete genome [KJ859682.1](#)
14. Usutu virus strain 2794, complete genome [KJ438767.1](#)
15. Usutu virus strain Meise H polyprotein gene, complete cds [JQ219843.1](#)
16. Usutu virus strain Vienna 2001 from Austria, complete genome [AY453411.1](#)
17. Usutu virus isolate Budapest, complete genome [EF206350.1](#)
18. Usutu virus strain Italia 2009, complete genome [JF266698.1](#)
19. Usutu virus strain Bologna 2009, complete genome [HM569263.1](#)
20. Usutu virus isolate ArB1803, complete genome KC754958.1

We obtained the amino acid sequence of our isolates by using EMBOSS software (available at <http://www.ebi.ac.uk/Tools/emboss/>), and we compared them using Clustal2.1.

Percent Identity Matrix:

```

1: 91418_Sassari2013  100.00  99.16
2: 62059_Sardegna2015  99.16  100.00

```

After that, we used BLAST software (<https://blast.ncbi.nlm.nih.gov/Blast>) to estimate the degree of identity at amino acid level among the strain 91418\_Sassari2013 and all the strains of USUV available in database.

This showed a Percent Identity Matrix of 99% with all USUV strains except:

"Berlin2015" [API68248.1](#) (98%) ; "Spain MB119/06" [AHA57377.1](#) (98%); "ArB1803" [AGP50649.1](#) (95%).

This is closely related with the Italian strains of USUV, in particular with:

"Bologna 2009" [AEF13245.1](#) ; "SE-10-Tm" [APC23728.1](#) ; "SE1-10-Tm" [APC23729.1](#).

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We found the presence of twelve unique mutations, N32S (M); F218V (E); L75P and K189E (NS1); A131G (NS2a); T64A (NS2b); E588G (NS3); T70I and L189M\* (\*related with 62059\_Sardegna2015) (NS4b); K402R; E560D and E738G (NS5).

The analysis of the genes *E* and *NS5* (involved in increase of virulence) and the complete genome sequence of the strain 91418\_Sassari2013 revealed several geography - and host - specific mutation.

The analysis was made by using the work of Engel *et al.* 2016 as a landmark.

In relation to the geography-specific mutations, we detected the specific mutation A120V (C) (also present in some Italian strains) that is fixed in all members of African lineages but lacks the mutations G320S (E); P376S (E) and A274T (NS5) are specific for African lineages.

There is the mutation A168T (E) specific for European lineages but lacks the mutation D67Y (E) specific for European lineages.

Concerning the country- and host-specific mutations, we found the presence of the mutation R195G (E) associated to Italian strains and mosquitoes hosts. On the other hand there was no presence of the mutation A161V (E) associated with African strains and mosquitoes. In addition we did not find the mutation I120N (M), specific for Germany and birds hosts and the mutations A91V (NS2a) and L46F (NS3) specific for bats.

Very important, are present the pathogenic mutations G302S (E) and D896E (NS5) related with increase of virulence in comparison with others *Flavivirus* (Sánchez *et al.* 2005; Gould *et al.* 2009; Chambers *et al.* 1998; Botha *et al.* 2008; McMinn PC 1997; Chin *et al.* 2007).

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### Isolate 62059\_Sardegna2015

The genome length for the isolate 62059\_Sardegna2015 is of 11009 nt that contains one large open reading frame (ORF) comprising nucleotides (nt) 97 to 10401 that encodes a unique polyprotein precursor of 3424 amino acid (aa).

The Percent Identity Matrix between the strain 62059\_Sardegna2015 and 91418\_Sassari2013 has been described previously.

Consequently, we used BLAST software (<https://blast.ncbi.nlm.nih.gov/Blast>) to estimate the degree of identity among the strain 62059\_Sardegna2015 and all the strains of USUV available in GenBank at nucleotide level.

This showed a Percent Identity Matrix of 99% with all USUV strains except African strains: "ArD101291" [KC754956.1](#) ; "HB81P08\_CAR\_1981" [KC754955.1](#) ; "ArD192495" [KC754957.1](#) ; "ArD19848" [KC754954.1](#) and "SAAR-1776\_South Africa" [AY453412.1](#) (98-97%) and the following European strains: "S.nebulosa-6004/NL/2016" [KY128482.1](#) (97%) and "Bonn" [KM659877.1](#) (97%);

The strain 62059\_Sardegna2015 is closely related with the Italian strains of USUV, in particular with:

"UR-10-Tm\_2015" [KX555624.1](#) ; "Bologna2009" [HM569263.1](#) ; "SE1-10-Tm\_2010" [KX555626.1](#). These were the same results of the strain 91418\_Sassari2013.

In this strain, the 5'-UTR is long 96 nt and its sequence is closely related with all European strains of USUV. However, more importantly, we have found the specific mutation G→T in position 8 which, until now, has been detected only in the strain "Bologna2009" (Gaibani *et al.* 2013).

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The 3'-UTR in this strain is long 611 nt and it was aligned with several sequences of USUV strains (strains list described previously), available in GenBank, to find unique or specific mutations. We found one specific mutation in position 5 T→C (already described before) and three unique mutations in position 124 T→C ; 204 G→A and 253 G→T.

Within the 3'-UTR it is present a short deletion motif of 16 nucleotides (**fig.4.8**) denominated “deleted motif 4” (DM4) (Engel *et al.* 2016) located in the stem loop SL-5 region (**fig. 4.9**) exactly as in the strain "HB81P08\_CAR\_1981". This was an extremely interesting discovery because this strain was isolated from a human patient presenting symptoms typical of USUV infection (Institut Pasteur de Dakar 1984; Nikolay *et al.* 2013).



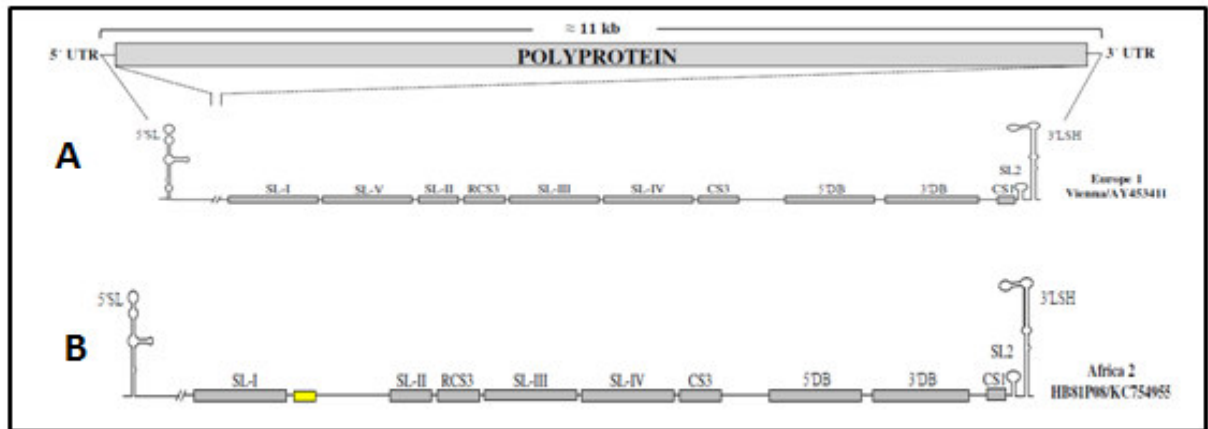
**Fig. 4.8** Alignment of several strains of USUV of 3'-UTR; There is a short deletion motif of 16 nucleotides exactly as in the strain "HB81P08\_CAR\_1981".

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**Fig. 4.9 (A)** Schematic representation of the patterns of RNA structures that occurred in the 5' and 3'-UTR of USUV. Reference model strain is “Vienna2001” (AY453411) (from Engel *et al.* 2016).

**(B)** Schematic representation of 16 nucleotides deletion in the stem loop SL-5 region.

In relation to the amino acid sequence, we used BLAST software (<https://blast.ncbi.nlm.nih.gov/Blast>) to estimate the degree of identity at amino acid level among the strain 62059\_Sardegna2015 and all the strains of USUV available in the database.

The result showed a Percent Identity Matrix of 99% with all USUV strains except:

"Berlin2015" [API68248.1](#) (98%) ; "Spain MB119/06" [AHA57377.1](#) (98%); "ArB1803" [AGP50649.1](#) (94%).

This is closely related with the Italian strains of USUV, in particular with:

"Bologna 2009" [AEF13245.1](#) ; "SE-10-Tm" [APC23728.1](#) ; "SE1-10-Tm" [APC23729.1](#).

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Are present nineteen unique mutations, S87P (M); K336E (E); I183T (NS1); Y57H and F123L (NS2a); R74C (NS2b); D211G and R619G (NS3); V158A ; L171P ; I179T and L189M\* (\*related with 91418\_Sassari2013) (NS4b); R29G ; R286G ; E313G ; T350A ; H499R and W706G (NS5).

In particular the mutation R619G (NS3) changes the cleavage site of the gene junction “NS3-NS4a”. Usually these are highly conserved but slight differences have been observed in all studied USUV ORFs (Engel *et al.* 2016). If these changes affect the virus assembly or RNA replication still remains to be clarified.

The analysis of the gene *E*, *NS5* and complete genome sequence of the strain 62059\_Sardegna2015 revealed several geography and host-specific mutation.

Even for this strain the analysis was made using the work of Engel *et al.* 2016 as a landmark.

The geography and host-specific mutations gave the same results of the strain 91418\_Sassari2013. However we found also a unique specific non synonymous mutation K336E (E) within the “Domain III” (DIII) of the E protein, which was far more interesting because DIII represents a receptor binding domain and a major determinant of virus cellular tropism (Chin *et al.* 2007).

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## Chapter 5

### Discussion

The first achievement of this research study was to find for the first time, through the monitoring on mosquitoes and birds, the evidence that USUV is circulating in Sardinia.

Moreover, the surveillance system confirms *C. pipiens* as the main vector of the virus in Sardinia, like in the rest of Italy, and its primary role in the circulation of USUV in the surveyed area. In addition, all the *C. pipiens*-positive pools were found only in the North Sardinia.

The results of entomological surveillance confirm also the gradual adaptation of the mosquito belonging to *Aedes* genus (and *Ochlerotatus*, *Aedes* sub-genus) to our climate and environment. This is an extremely dangerous fact for future health threat because *Aedes* mosquito is the main vector known for *Zika* virus (ZIKV).

It is important to also note that only one bird tested positive for USUV in passive surveillance and no one in active surveillance.

Regarding the fact that some positive samples of USUV were found by us (67279/1; 80158; 54860/1; 59265; 71253/2 and 81088) and the difficulty to confirm the positivity even by traditional PCR and sequencing, we think that these isolates may have a high genetic variability in comparison to the others strains of USUV. This is the reason why we cannot amplify through traditional PCR, or as an alternative maybe, these samples of USUV are false positive USUV, due to the wide variety of “mosquito-only flavivirus” constitutionally present in mosquitoes. In addition, *Flavivirus* typically have very similar and well preserved

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genomic sequences, which can give a cross-reactivity between them (Gaibani *et al.* 2010; Calzolari *et al.* 2012; Grisenti *et al.* 2015; Roiz *et al.* 2012; Vázquez *et al.* 2012).

The last hypothesis is that the samples are degraded. However, these are hypotheses that have to be verified in the future using more advanced methods like Next Generation Sequencing (NGS).

In relation to the positive sample also confirmed in traditional PCR, we determined the full-length nucleotide sequence of the isolates 91418 and 62059/4 that designed respectively strain “91418\_Sassari2013” and strain “62059\_Sardegna2015” through the technique of “Primer-Walking”, with a new protocol developed by us to allow to overcome the problem of the small amount of sample, which is typical with mosquitoes pool sample.

Subsequently, we processed these samples for phylogenetic analysis and compared them with others sequences of USUV present in the database. This was done to investigate mutations associated with increased virulence or associated with geography- or host-adaptation.

The local strains are closely related to the Italian strains and in particular with “Bologna2009”. This is particularly interesting because “Bologna2009” has been the first strain isolate from a human patient with neurological symptoms (Gaibani *et al.* 2009). However, it is noted that they have their own molecular characteristics in comparison to Italian strains, like, for example, the deletions in 3'-UTR, which until now have not been detected in Italian strains.

Phylogenetic results likely confirm the theory that the genetic diversity of USUV is shaped primarily by *in situ* evolution (enzootic maintenance) (Engel *et al.* 2016), due to the fact that

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USUV circulates in multiple areas that are separated from each other by geographic barriers such as climate, vegetation, different host species, and other unknown ecological conditions. It is important to stress the fact that Sardinia is an island whose environment is protected against external influence.

It is significant to underline that the strains 91418\_Sassari2013 and 62059\_Sardegna2015 are both characterized by having the geography- and host-specific mutation R195G (E) related to Italian strains and mosquito host and the pathogenic mutations G302S (E) and D896E (NS5). This is particularly important because many studies of the closely related flavivirus WNV have shown that substitutions, in virtually equivalent positions in these two genes, were associated with variation in the capacity of WNV to invade the central nervous system (CNS) of laboratory mice under experimental conditions (Sánchez *et al.* 2005; Gould EA and Higgs S 2009; Chambers *et al.* 1998).

In fact, in relation to the pathogenic mutation G302S (E), specific amino acid substitutions, within domain DIII of the E protein, have been implicated as mediators/moderators of virus infectivity, virulence, antigenicity and the capacity to escape from neutralizing antibodies for WNV (Gould EA and Higgs S 2009; Chu *et al.* 2005). Interestingly, similar amino acid substitutions in the E domain (DIII) were found in DENV-2 isolates in Southern-Asia, that were associated with cases of human encephalitis, whether or not they were also associated with classical clinical manifestations of dengue infection (Zang *et al.* 2010). In the strain 62059\_Sardegna2015 we found also a unique non synonymous mutation K336E (E) with amino acid charge changes within domain DIII.

The flavivirus NS5 protein encodes the RNA dependent RNA polymerase which is a critical component of the virus replication complex.

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With regards to the mutation D896E (NS5), associated with viral replication efficiency and neuro-invasive capacity in certain strains of JEV and WNV (Gaibani *et al.* 2012), we detected, within the NS5 protein, four unique non synonymous mutations R29G; R286G; E313G and T350A in the strain 62059\_Sardegna2015 and one unique non synonymous mutation E738G in the strain 91418\_Sassari2013. Substitutions in the preserved domain of this polymerase can impact viral replication efficiency via perturbation of the viral replication complex. In fact, a single amino acid charge has been found to influence WNV replication in different hosts (Van Slyke *et al.* 2012).

We also found a putative large deletion in 3'-UTR in the strain 91418\_Sassari2013 and a short deletion of 16 nucleotides located in the stem loop SL-5 region in the strain 62059\_Sardegna2015 and this might alter these secondary structures and thereby influence virus infectivity. Actually, it has been shown that the variation in this region may have evolved as a function of dengue virus (DENV) transmission and replication in different mosquitoes and nonhuman primate/human host cycles (Shurtleff *et al.* 2001).

The 3'-UTR is important for *Flavivirus* replication and virulence determination, as the formation of secondary structures serve as cis-acting elements during RNA transcription (Hurrelbrink RJ and McMinn PC 2003).

In the strain 62059\_Sardegna2015 are also present three unique nucleotide mutations 124 T→C ; 204 G→A ; 253 C→T and previous studies with WNV have demonstrated that single nucleotide substitutions in the 3'-UTR region can affect RNA processing and replication, as well as polyprotein synthesis and maturation during the replication cycle (Turner *et al.* 2004; Davis *et al.* 2007).

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In conclusion, we found some significant molecular features in our strains, in particular in the strain 62059\_Sardegna2015, most of them associated with the increase of virulence and ability to infect mammals including humans. However further studies will be required to better understand the effects of these strain-specific nucleotide and amino acid substitutions, and in particular comparative pathogenesis studies on different USUV strains using animal models will be essential.

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PhD Thesis in Life Sciences and Biotechnologies Curriculum Microbiology and Immunology

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## Bibliography

1. **Adam, F.; Digoutte, J.-P.** 2014. Virus d’Afrique (base de données). In *Centre Collaborateur OMS de Référence et de Recherche Pour les Arbovirus et les Virus de Fièvre hémorragiques (CRORA)*; Institut Pasteur de Dakar: Dakar, Senegal. Available online: <http://www.pasteur.fr/recherche/banques/CRORA/>
2. **Allering L, Jost H, Emmerich P, Gunther S, Lattwein E, et al.** 2012. Detection of Usutu virus infection in a healthy blood donor from south-west Germany, 2012. *Euro Surveill* 17: pii: 20341.
3. **Ashraf U, Ye J, Ruan X, Wan S, Zhu B, Cao S.** 2015. Usutu virus: an emerging flavivirus in Europe. *Viruses*. 7(1):219-38.
4. **Bakonyi, T.; Gould, E.A.; Kolodziejek, J.; Weissenböck, H.; Nowotny, N.** 2004. Complete genome analysis and molecular characterization of Usutu virus that emerged in Austria in 2001: Comparison with the South African strain SAAR-1776 and other flaviviruses. *Virology* 328, 301–310.
5. **Bakonyi, T.; Lussy, H.; Weissenböck, H.; Hornyák, A.; Nowotny, N.** 2005. *In vitro* host-cell susceptibility to Usutu virus. *Emerg. Infect. Dis.* 11, 298–301.
6. **Bakonyi, T.; Erdélyi, K.; Ursu, K.; Ferenczi, E.; Csörge, T.; Lussy, H.; Chvala, S.; Bukovsky, C.; Meister, T.; Weissenböck, H.; et al.** 2007. Emergence of Usutu virus in Hungary. *J. Clin. Microbiol.* 45, 3870–3874.
7. **Becker N, Zgomba M, Petric D, Dahl C, Boase C, et al.** 2010. Mosquitoes and their Control. 2nd Edition. New York: Kluwer Academic Publishers. 498 p.

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“CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF *USUTU* VIRUS ISOLATES IN SARDINIA”

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8. **Becker, N.; Jöst, H.; Ziegler, U.; Eiden, M.; Höper, D.; Emmerich, P.; Fichet-Calvet, E.; Ehichioya, D.U.; Czajka, C.; Gabriel, M.; et al.** 2012. Epizootic emergence of Usutu virus in wild and captive birds in Germany. *PLoS One* 7, e32604.
9. **Ben Hassine, T.; de Massis, F.; Calistri, P.; Savini, G.; BelHaj Mohamed, B.; Ranen, A.; di Gennaro, A.; Sghaier, S.; Hammami, S.** 2014. First detection of co-circulation of West Nile and Usutu viruses in equids in the south-west of Tunisia. *Transbound. Emerg. Dis.* 61, 385–389.
10. **Botha EM, Markotter W, Wolfaardt M, Paweska JT, Swanepoel R, et al.** 2008. Genetic determinants of virulence in pathogenic lineage 2 West Nile virus strains. *Emerg Infect Dis* 14: 222–230.
11. **Blackwell, J.L., Brinton, M.A.,** 1995. BHK cell proteins that bind to the 3' stem-loop structure of the West Nile Virus genome RNA. *J. Virol.* 69, 5650–5658.
12. **Blackwell, J.L., Brinton, M.A.,** 1997. Translation elongation factor-1 alpha interacts with the 3' stem-loop region of West Nile virus genomic RNA. *J. Virol.* 71, 6433–6444.
13. **Blázquez, A.B.; Escribano-Romero, E.; Merino-Ramos, T.; Saiz, J.S.; Martín-Acebes, M.A.** 2013. Infection with Usutu virus induces an autophagic response in mammalian cells. *PLoS Negl. Trop. Dis* 7, e2509.
14. **Brinton, M.A., Fernandez, A.V., Dispoto, J.H.,** 1986. The 3'-nucleotides of flavivirus genomic RNA form a conserved secondary structure. *Virology* 153, 113–121.
15. **Buckley, A.; Dawson, A.; Gould, E.A.** 2006. Detection of seroconversion to West Nile virus, Usutu virus and Sindbis virus in UK sentinel chickens. *Virol. J.* 3, e71.
16. **Busquets, N.; Alba, A.; Allepuz, A.; Aranda, C.; Ignacio Nuñez, J.** 2008. Usutu virus sequences in *Culex pipiens* (Diptera: *Culicidae*), Spain. *Emerg. Infect. Dis.* 14, 861–863.

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 PhD Thesis in Life Sciences and Biotechnologies Curriculum Microbiology and Immunology  
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17. **Cadar, D.; Becker, N.; Campos Rde, M.; Börstler, J.; Jöst, H.; Schmidt-Chanasit, J.** 2014. Usutu virus in bats, Germany, 2013. *Emerg. Infect. Dis.* 20, 1771–1773.
18. **Calisher, C.H., Karabatos, N., Dalrymple, J.M., Shope, R.E., Porterfield, J.S., Westerway, E.G., Brandt, W.E.,** 1989. Antigenic relationships between flaviviruses as determined by cross-neutralisation tests with polyclonal antisera. *J. Gen. Virol.* 70, 37–43.
19. **Calisher CH, Gould EA** 2003. Taxonomy of the virus family *Flaviviridae*. *Adv Virus Res* 59: 1-19.
20. **Calzolari, M.; Bonilauri, P.; Bellini, R.; Albieri, A.; Defilippo, F.; Maioli, G.; Galletti, G.; Gelati, A.; Barbieri, I.; Tamba, M.; et al.** 2010. Evidence of simultaneous circulation of West Nile and Usutu viruses in mosquitoes sampled in Emilia-Romagna region (Italy) in 2009. *PLoS One* 5, e14324.
21. **Calzolari, M.; Gaibani, P.; Bellini, R.; Defilippo, F.; Pierro, A.; Albieri, A.; Mailoli, G.; Luppi, A.; Rossini, G.; Balzani, A.; et al.** 2012. Mosquito, bird, and human surveillance of West Nile and Usutu viruses in Emilia-Romagna region (Italy) in 2010. *PLoS One* 7, e38058.
22. **Calzolari M, Zé-Zé L, Růžek D, Vázquez A et al.** 2012. Detection of mosquito-only flaviviruses in Europe. *J Gen Virol.* 93(Pt 6):1215-25.
23. **Calzolari, M.; Bonilauri, P.; Bellini, R.; Albieri, A.; Defilippo, F.; Tamba, M.; Tassinari, M.; Gelati, A.; Cordioli, P.; Angelini, P.; et al.** 2013. Usutu virus persistence and West Nile virus inactivity in the Emilia-Romagna region (Italy) in 2011. *PLoS One* 8, e63978.

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“CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF *USUTU* VIRUS ISOLATES IN SARDINIA”

PhD Thesis in Life Sciences and Biotechnologies Curriculum Microbiology and Immunology

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24. **Calzolari M, Pautasso A, Montarsi F et al.** 2015. West Nile Virus Surveillance in 2013 via Mosquito Screening in Northern Italy and the Influence of Weather on Virus Circulation. *PLoS One*. 10(10):e0140915.
25. **Cavrini F, Gaibani P, Longo G, Pierro AM, Rossini G, Bonilauri P, et al.** 2009. Usutu virus infection in a patient who underwent orthotopic liver transplantation, Italy, August-September 2009. *Euro Surveill*. 14 (50).
26. **Cavrini F, Della Pepa ME, Gaibani P, Pierro AM, Rossini G, et al.** 2011. A rapid and specific real-time RT-PCR assay to identify Usutu virus in human plasma, serum, and cerebrospinal fluid. *J Clin Virol* 50: 221–223.
27. **CDC Guidelines for Surveillance, Prevention, & Control.** 2001. 3<sup>rd</sup> revision. Fort Collins CO. Available: <http://www.cdc.gov/ncidod/dvbid/westnile/resources/wnv-guidelines-aug-2003.pdf>.
28. **Chaintoutis, S.C.; Dovas, C.I.; Papanastassopoulou, M.; Gewehr, S.; Danis, K.; Beck, C.; Lecollinet, S.; Antalis, V.; Kalaitzopoulous, S.; Panagiotopoulos, T.; et al.** 2014. Evaluation of a West Nile virus surveillance and early warning system in Greece, based on domestic pigeons. *Comp. Immunol. Microbiol. Infect. Dis.* 37, 131–141.
29. **Chambers TJ, Halevy M, Nestorowicz A, Rice CM, Lustig S** 1998. West Nile virus envelope proteins: nucleotide sequence analysis of strains differing in mouse neuroinvasiveness. *J Gen Virol* 79: 2375–2380.
30. **Chen, C.J., Kuo, M.D., Chien, L.J., Hsu, S.L., Wang, Y.M., Lin, J.H.,** 1997. RNA-protein interactions: involvement of NS3, NS5, and 3' noncoding regions of Japanese Encephalitis virus genomic RNA. *J. Virol.* 71, 3466–3473.
31. **Chevalier, V.; Reynaud, P.; Lefrancois, T.; Durand, B.; Baillon, F.; Balanca, G.; Gaidet, N.; Mondet, B.; Lancelot, R.** 2009. Predicting West Nile virus seroprevalence in wild birds in Senegal. *Vector Borne Zoonotic Dis.* 9, 589–596.

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PhD Thesis in Life Sciences and Biotechnologies Curriculum Microbiology and Immunology

University of Sassari

32. **Chin JF, Chu JJ, Ng ML** 2007. The envelope glycoprotein domain III of dengue virus serotypes 1 and 2 inhibit virus entry. *Microbes Infect* 9: 1–6.
33. **Chu JJ, Rajamanonmani R, Li J, Bhuvanakantham R, Lescar J, et al.** 2005. Inhibition of West Nile virus entry by using a recombinant domain III from the envelope glycoprotein. *J Gen Virol* 86: 405–412.
34. **Chvala S, Kolodziejek J, Nowotny N, Weissenbock H** 2004. Pathology and viral distribution in fatal Usutu virus infections of birds from the 2001 and 2002 outbreaks in Austria. *J Comp Pathol* 131: 176–185.
35. **Cornet, M.; Robin, Y.; Chateau, R.; Heme, G.; Adam, C.; Valade, M.; Le Gonidec, G.; Jan, C.; Renaudet, J.; Dieng, P.Y.; et al.** 1979. Isolement d'arbovirus au Sénégal Oriental a partir de moustiques (1972–1977) et notes sur l'épidémiologie des virus transmis par les Aedes en particulier du virus amaril. *Cahiers ORSTOM. Sér. Entomologieméd. Parasitol.* 17, 149–163.
36. **Davis WG, Blackwell JL, Shi PY, Brinton MA** 2007. Interaction between the cellular protein eEF1A and the 3'-terminal stem-loop of West Nile virus genomic RNA facilitates viral minus-strand RNA synthesis. *J Virol* 81: 10172–10187.
37. **Engel D, Jöst H, Wink M et al.** 2016. Reconstruction of the Evolutionary History and Dispersal of Usutu Virus, a Neglected Emerging Arbovirus in Europe and Africa. *MBio.* 7(1):e01938-15.
38. **Felsenstein J.** 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791.

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“CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF *USUTU* VIRUS ISOLATES IN SARDINIA”

PhD Thesis in Life Sciences and Biotechnologies Curriculum Microbiology and Immunology

University of Sassari

39. **Figuerola, J.; Soriguer, R.; Rojo, G.; Gómez Tejedor, C.; Jimenez-Clavero, M.A.** 2007. Seroconversion in wild birds and local circulation of West Nile virus, Spain. *Emerg. Infect. Dis.* 13, 1915–1917.
40. **Gaibani P, Pierro AM, Cavrini F, Rossini G, Landini MP, et al.** 2010. Falsepositive transcription-mediated amplification assay detection of West Nile virus in blood from a patient with viremia caused by an Usutu virus infection. *J Clin Microbiol* 48: 3338–3339.
41. **Gaibani P, Pierro A, Alicino R, Rossini G, Cavrini F, et al.** 2012. Detection of Usutu-virus-specific IgG in blood donors from northern Italy. *Vector Borne Zoonotic Dis* 12: 12431–12433.
42. **Garigliany, M.M.; Marlier, D.; Tenner-Racz, K.; Eiden, M.; Cassart, D.; Gandar, F.; Beer, M.; Schmidt-Chanasit, J.; Desmecht, D.** 2014. Detection of Usutu virus in a bullfinch (*Pyrrhula pyrrhula*) and a great spotted woodpecker (*Dendrocopos major*) in north-west Europe. *Vet. J.* 199, 191–193.
43. **Gould EA, Higgs S** 2009. Impact of climate change and other factors on emerging arbovirus diseases. *Trans R Soc Trop Med Hyg* 103: 109–121.
44. **Grange, T., Bouloy, M., Girard, M.,** 1985. Stable secondary structures at the 3'-end of the genome of yellow fever virus (17 D vaccine strain). *FEBS Letters* 188, 159–163.
45. **Gratz NG** 2006. *Vector- and Rodent-Borne Diseases in Europe and North America. Distribution, Burden PublicHealth, eds. and Control.* New York: Cambridge University Press. 393 p.
46. **Grisenti M, Vázquez A, Herrero L. et al.** 2014. Wide detection of Aedes flavivirus in north-eastern Italy a European hotspot of emerging mosquito-borne diseases. *J Gen Virol.* 96(Pt 2):420-30.

Roberto Bechere

“CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF *USUTU* VIRUS ISOLATES IN SARDINIA”  
 PhD Thesis in Life Sciences and Biotechnologies Curriculum Microbiology and Immunology  
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47. **Gritsun, T.S., Venugopal, K., Zanotto, deA., Mikhailov, M., Sall, E., Holmes, E.C., Polkinghorne, I., Frolova, T.V., Pogodina, V.V., Lashkevich, V.A., Gould, E.A.,** 1997. Complete sequence of two tick-borne flaviviruses isolated from Siberia and the UK: analysis and significance of the 5' and 3'-UTRs. *Virus Res.* 49, 27–39.
48. **Hahn, C.S., Hahn, Y.S., Rice, C.M., Lee, E., Dalgarno, L., Strauss, E.G., Strauss, J.H.,** 1987. Conserved elements in the 3' untranslated region of flavivirus RNAs and potential cyclization sequences. *J. Mol. Biol.* 198, 33–41.
49. **Heinz, F.X., Collett, M.S., Purcell, R.H., Gould, E.A., Howard, C.R., Houghton, M., Moormann, R.J., Rice, C.M., Thiel, H.J.,** 2000. Family *Flaviviridae*. In: van Regenmortel, M.H.V., Faquet, C.M., Bishop, D.H.L. (Eds.), *Virus Taxonomy, Seventh International Committee for the Taxonomy of Viruses*. Academic Press, San Diego, pp. 859–878.
50. **Höfle, U.; Gamino, V.; de Mera, I.G.; Mangold, A.J.; Ortíz, J.A.; de la Fuente, J.** 2013. Usutu virus in migratory song thrushes, Spain. *Emerg. Infect. Dis.* 19, 1173–1175.
51. **Holmes EC** 2003. Patterns of intra- and interhost nonsynonymous variation reveal strong purifying selection in dengue virus. *J Virol* **77**: 11296–11298.
52. **Hubálek Z** 2008. Mosquito-borne viruses in Europe. *Parasitol Res* 103: S29–S43.
53. **Hubálek, Z.; Halouzka, J.; Juricová, Z.; Sikutová, S.; Rudolf, I.; Honza, M.; Janková, J.; Chytil, J.; Marec, F.; Sitko, J.** 2008. Serologic survey of birds for West Nile flavivirus in southern Moravia (Czech Republic). *Vector Borne Zoonotic Dis.* 8, 659–666.

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54. **Hubálek, Z.; Wegner, E.; Halouzka, J.; Tryjanowski, P.; Jerzak, L.; Sikutová, S.; Rudolf, I.; Kruszcwicz, A.G.; Jaworski, Z.; Włodarczky, R.** 2008. Serologic survey of potential vertebrate hosts for West Nile virus in Poland. *Viral Immunol.* 21, 247–253.
55. **Hubálek, Z.; Rudolf, I.; Čapek, M.; Bakonyi, T.; Betášová, L.; Nowotny, N.** 2014. Usutu virus in blackbirds (*Turdus merula*), Czech Republic, 2011–2012. *Transbound. Emerg. Dis.* 61, 273–276.
56. **Hurrelbrink RJ, McMinn PC** 2003. Molecular determinants of virulence: the structural and functional basis for flavivirus attenuation. *Adv Virus Res* 60:1–42.
57. **Institut Pasteur de Dakar** 1984. Rapport sur le fonctionnement technique de l’Institut Pasteur de Dakar. Dakar: Institut Pasteur de Dakar; 1982–1984.
58. **Karabatsos, N.** 1985. Usutu virus. In *International Catalogue of Arboviruses Including Certain other Viruses of Vertebrates*, 3rd ed.; The American Society of Tropical Medicine and Hygiene: San Antonio, TX, USA, pp. 1059–1060.
59. **Khakpoor, A.; Panyasrivanit, M.; Wikan, N.; Smith, D.R.** 2009. A role for autophagolysosomes in dengue virus 3 production in HepG2 cells. *J. Gen. Virol.* 90, 1093–1103.
60. **Kramer LD, Styer LM, Ebel GD** 2008. A global perspective on the epidemiology of West Nile virus. *Annu Rev Entomol.* 53: 61–81.
61. **Kuno, G.; Chang, G.J.; Tsuchiya, K.R.; Karabatsos, N.; Cropp, C.B.** 1998. Phylogeny of the genus *Flavivirus*. *J. Virol.* 72, 73–83.

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PhD Thesis in Life Sciences and Biotechnologies Curriculum Microbiology and Immunology  
University of Sassari

62. **Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG.** 2007. Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947-2948.
63. **Lelli, R.; Savini, G.; Teodori, L.; Filipponi, G.; Di Gennaro, A.; Leone, A.; Gialleonardo, L.; Venturi, L.; Caporate, V.** 2008. Serological evidence of Usutu virus occurrence in north-eastern Italy. *Zoonoses Public Health* 55, 361–367.
64. **Li, J.K.; Liang, J.J.; Liao, C.L.; Lin, Y.L.** 2012. Autophagy is involved in the early step of Japanese encephalitis virus infection. *Microbes Infect.* 14, 159–168.
65. **Linke, S.; Niedrig, M.; Kaiser, A.; Ellerbrok, H.; Müller, K.; Müller, T.; Conraths, F.J.; Mühle, R.U.; Schmidt, D.; Köppen, U.; et al.** 2007. Serologic evidence of West Nile virus infections in wild birds captured in Germany. *Am. J. Trop. Med. Hyg.* 77, 358–364.
66. **Manarolla, G.; Bakonyi, T.; Gallazzi, D.; Crosta, L.; Weissenböck, H.; Dorrestein, G.M.; Nowotny, N.** 2010. Usutu virus in wild birds in northern Italy. *Vet. Microbiol.* 141, 159–163.
67. **Mandl, C.W., Holzmann, H., Kunz, K., Heinz, F.X.,** 1993. Complete genomic sequence of Powassan Virus: evaluation of genetic elements in tick-borne versus mosquito-borne flaviviruses. *Virology* 194, 173–184.
68. **Mani, P.; Rossi, G.; Perrucci, S.; Bertini, S.** 1998. Mortality of *Turdus merula* in Tuscany. *Sel. Vet.* 8, 749–753.
69. **McMinn PC** 1997. The molecular basis of virulence of the encephalitogenic flaviviruses. *J Gen Virol* 78: 2711–2722.

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“CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF *USUTU* VIRUS ISOLATES IN SARDINIA”  
 PhD Thesis in Life Sciences and Biotechnologies Curriculum Microbiology and Immunology  
 University of Sassari

70. **Miller, S.; Krijnse-Locker, J.** 2008. Modification of intracellular membrane structures for virus replication. *Nat. Rev. Microbiol* 6, 363–374.
71. **Mizushima N, Levine B, Cuervo AM, Klionsky DJ** 2008. Autophagy fights disease through cellular self-digestion. *Nature* 451: 1069–1075.
72. **Mohan, P.M., Padmanabhan, R.,** 1991. Detection of stable secondary structure at the 3' terminal of dengue virus type 2 RNA. *Gene* 108, 837–838.
73. **Nikolay, B.; Diallo, M.; Boye, C.S.; Sall, A.A.** 2011. Usutu virus in Africa. *Vector Borne Zoonotic Dis.* 11, 1417–1423.
74. **Nikolay, B.; Dupressoir, A.; Firth, C.; Faye, O.; Boye, C.S.; Diallo, M.; Sall, A.A.** 2013. Comparative full length genome sequence analysis of Usutu virus isolates from Africa. *Viol. J.* 10, e217.
75. **Orvedahl A, Levine B** 2008. Viral evasion of autophagy. *Autophagy* 4: 280–285.
76. **Paniz-Mondolfi AE, Villamil-Gómez WE, Rodríguez-Morales AJ** 2016. Usutu virus infection in Latin America: A new emerging threat. *Travel Med Infect Dis.* 14(6):641-643.
77. **Pecorari M, Longo G, Gennari W, Grottola A, Sabbatini A, Tagliazucchi S, et al.** 2009. First human case of Usutu virus neuroinvasive infection, Italy, August-September 2009. *Euro Surveill.* 14(50).
78. **Poidinger, M.; Hall, R.A.; Mackenzie, J.S.** 1996. Molecular characterization of the Japanese encephalitis serocomplex of the *Flavivirus* genus. *Virology* 218, 417–421.

Roberto Bechere

“CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF *USUTU* VIRUS ISOLATES IN SARDINIA”

PhD Thesis in Life Sciences and Biotechnologies Curriculum Microbiology and Immunology

University of Sassari

79. **Pradier S, Lecollinet S, Leblond A.** 2012. West Nile virus epidemiology and factors triggering change in its distribution in Europe. *Rev Sci Tech.* 31(3):829-44.
80. **Proutski V, Gritsun TS, Gould EA, Holmes EC.** 1999. Biological consequences of deletions within the 3'-untranslated region of flaviviruses may be due to rearrangements of RNA secondary structure. *Virus Res.* 64(2):107-23.
81. **Rice, C.M., Lenches, E.M., Eddy, S.R., Shin, S.J., Sheets, R.L., Strauss, J.H.,** 1985. Nucleotide sequence of yellow fever virus: Implications for flavivirus gene expression and evolution. *Science* 229, 726–733.
82. **Rice, C.M., Strauss, E.G., Strauss, J.H.,** 1986. Structure of the flavivirus genome. In: Schlesinger, S., Schlesinger, M.J. (Eds.), *The Togaviridae and Flaviviridae*. Plenum, New York, pp. 279–326.
83. **Rossi R, Rocchigiani AM, Manunta D, Foxi C, Bechere R, Cappai S, Portanti O, Satta G, Monaco F, Puggioni G.** 2016. Entomological surveillance for West Nile and Usutu viruses in Sardinia (Italy) during 2015. *International Journal of Infectious Diseases*, Volume 53, Supplement, Page 160.
84. **Roiz D, Vázquez A, Rosso F. et al.** 2012. Detection of a new insect flavivirus and isolation of *Aedes flavivirus* in Northern Italy. *Parasit Vectors.* 5:223.
85. **Saitou N. and Nei M.** 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406-425.
86. **Sanchez MD, Pierson TC, McAllister D, Hanna SL, et al.** 2005. Characterization of neutralizing antibodies to West Nile virus. *Virology* 336: 70–82.

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“CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF *USUTU* VIRUS ISOLATES IN SARDINIA”  
 PhD Thesis in Life Sciences and Biotechnologies Curriculum Microbiology and Immunology  
 University of Sassari

87. **Sánchez-Seco MP, Rosario D, Domingo C, Hernández L, Valdés K, Guzmán MG, Tenorio A.** 2005. Generic RT-nested-PCR for detection of flaviviruses using degenerated primers and internal control followed by sequencing for specific identification. *J Virol Methods*. 126(1-2):101-9.
88. **Savage HM, Strickman D** 2004. The genus and subgenus categories within Culicidae and placement of *Ochlerotatus* as a subgenus of *Aedes*. *J Am Mosq Control Assoc* 20: 208–214.
89. **Savini G, Monaco F, Terregino C, Di Gennaro A, Bano L, et al.** 2011. Usutu virus in Italy: an emergence or a silent infection? *Vet Microbiol* 151: 264–274.
90. **Schaffner E, Angel G, Geoffroy B, Hervy JP, Rhaïem A, et al.** 2001. *The Mosquitoes of Europe*. Paris: IRD editions.
91. **Shurtleff AC, Beasley DW, Chen JJ, Ni H, Suderman MT, Wang H, Xu R, Wang E, Weaver SC, Watts DM, Russell KL, Barrett AD** 2001. Genetic variation in the 3' non-coding region of dengue viruses. *Virology* 281:75–87.
92. **Severini F, Toma L, Di Luca M, Romi R** 2009. Le zanzare italiane: generalità e identificazione degli adulti (Diptera, Culicidae). *Fragmenta entomologica* 41: 213–372.
93. **Shi, P.Y., Brinton, M.A., Veal, J.M., Zhong, Y.Y., Wilson, W.D.,** 1996. Evidence for existence of a pseudoknot structure at the 3' terminal of the flavivirus genomic RNA. *Biochemistry* 35, 4222–4230.
94. **Steinmetz, H.W.; Bakonyi, T.; Chvala, S.; Weissenböck, H.; Eulenberger, U.; Hatt, J.M.; Hoop, R.; Nowotny, N.** 2007. Emergence of Usutu virus in Switzerland. In *Proceedings of the 43rd International Symposium on Diseases of Zoo and Wild Animals*, Edinburgh, UK, 16–20 May 2007; pp. 129–131.

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“CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF *USUTU* VIRUS ISOLATES IN SARDINIA”  
 PhD Thesis in Life Sciences and Biotechnologies Curriculum Microbiology and Immunology  
 University of Sassari

95. **Steinmetz HW, Bakonyi T, Weissenböck H, Hatt JM, Eulenberger U, Robert N, Hoop R, Nowotny N.** 2011. Emergence and establishment of Usutu virus infection in wild and captive avian species in and around Zurich, Switzerland genomic and pathologic comparison to other central European outbreaks. *Vet Microbiol.* 148(2-4):207-12.
96. **Stojanovich CJ, Scott HG** 1997. Mosquitoes of the Italian biogeographical area which includes the Republic of Malta, the French Island of Corsica and all of Italy except the far northern Provinces. USA: Ed. Stojanovich CJ & Scott HG. 199 p.
97. **Van Slyke GA, Ciota AT, Willsey GG, Jaeger J, Shi PY, Kramer LD** 2012. Point mutations in the West Nile virus (Flaviviridae; flavivirus) RNA-dependent RNA polymerase alter viral fitness in a host-dependent manner in vitro and in vivo. *Virology* 427:18–24.
98. **Vázquez, A.; Ruiz, S.; Herrero, L.; Moreno, J.; Molero, F.; Magallanes, A.; Sánchez-Seco, M.P.; Figuerola, J.; Tenorio, A.** 2011. West Nile and Usutu viruses in mosquitoes in Spain, 2008–2009. *Am. J. Trop. Med. Hyg.* 85, 178–181.
99. **Vázquez A, Sánchez-Seco MP, Palacios G. et al.** 2012. Novel flaviviruses detected in different species of mosquitoes in Spain. *Vector Borne Zoonotic Dis.* 12(3):223-9.
100. **Vázquez, A.; Jiménez-Clavero, M.A.; Franco, L.; Donoso-Mantke, O.; Sambri, V.; Niedrig, M.; Zeller, H.; Tenorio, A.** 2014. Usutu virus–potential risk of human disease in Europe. *Euro. Surveill.* 16, e19935.
101. **Wallner, G., Mandl, C.W., Kunz, C., Heinz, F.X.,** 1995. The flavivirus 3'-noncoding region: extensive size heterogeneity independent of evolutionary relationships among strains of Tick-Borne Encephalitis Virus. *Virology* 213, 169–178.

Roberto Bechere

“CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF *USUTU* VIRUS ISOLATES IN SARDINIA”

PhD Thesis in Life Sciences and Biotechnologies Curriculum Microbiology and Immunology

University of Sassari

102. **Wang, E., Weaver, S.C., Shope, R.E., Tesh, R.B., Watts, D.M., Barrett, A.D.,** 1996. Genetic variation in yellow fever virus: duplication in the 3' noncoding region of strains from Africa. *Virology* 225, 274–281.
103. **Weaver SC, Reisen WK** 2010. Present and future arboviral threats. *Antiviral Res* 85: 328–345.
104. **Weissenböck H, Kolodziejek J, Fragner K, Kuhn R, Pfeiffer M** 2003. Usutu virus activity in Austria, 2001–2002. *Microbes Infect* 5: 1132–1136.
105. **Weissenböck, H.; Bakonyi, T.; Chvala, S.; Nowotny, N.** 2004. Experimental Usutu virus infection of suckling mice causes neuronal and glial cell apoptosis and demyelination. *Acta Neuropathol* 108, 453–460.
106. **Weissenböck, H.; Bakonyi, T.; Rossi, G.; Mani, P.; Nowotny, N.** 2013. Usutu virus, Italy, 1996. *Emerg. Infect. Dis.* 19, 274–277.
107. **Yu, C.Y.; Hsu, Y.W.; Liao, C.L.; Lin, Y.L.** 2006. Flavivirus infection activates the XBP1 pathway of the unfolded protein response to facilitate replication and immune evasion. *J. Virol* 80, 11868–11880.
108. **Tamba M, Bonilauri P, Bellini R, Calzolari M, Albieri A, et al.** 2011. Detection of Usutu virus within a West Nile virus surveillance program in Northern Italy. *Vector Borne Zoonotic Dis* 11: 551–557.
109. **Tamura K., Nei M., and Kumar S.** 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences (USA)* 101:11030-11035.

Roberto Bechere

“CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF *USUTU* VIRUS ISOLATES IN SARDINIA”

PhD Thesis in Life Sciences and Biotechnologies Curriculum Microbiology and Immunology

University of Sassari

110. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30: 2725–2729.
  
111. **Thurner C, Witwer C, Hofacker IL, Stadler PF** 2004. Conserved RNA secondary structures in Flaviviridae genomes. *J Gen Virol* 85: 1113–1124.
  
112. **Zhang S, Bovshik EI, Maillard R, Gromowski GD, Volk DE, et al.** 2010. Role of BC loop residues in structure, function and antigenicity of the West Nile virus envelope protein receptor-binding domain III. *Virology* 403: 85–91.

Roberto Bechere

“CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF *USUTU* VIRUS ISOLATES IN SARDINIA”  
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Technical Appendix (**Table S1**)

Primers used for the full-length amplification of *Usutu* virus

- 1) UsuvF1deg **AGWYGTTBGYCTGYGTGAGC** / Usuv327R **TGCCGTGGTCTTGTTGATGC** (327 bp)
- 2) Usuv232F **TTTGTGCTGGCCTTGATGAC** / Usuv1246R **CGTACGTGCCTCAGCTCTC** (934 bp)
- 3) Usuv1124F **CACGAACCTGGCTGAAGTGA** / Usuv2353R **TGATCCAGGACATGCCACCG** (1229 bp)
- 4) Usuv2295F **GTACATCAGGTCTTTGGAGG** / Usuv4693R **TGTAACGGCTCATGATACGG** (2398 bp)
- 5) Usuv4578F **TGGCTCACTGTCAAGTACGC** / Usuv5509R **CAATGTAGCCTCGTGCTGCTAT** (931 bp)
- 6) Usuv5443F **ACCTCTTTGTGATGGATGAG** / Usuv6935R **GTTCTCTCTAACATTCCGTA** (1492 bp)
- 7) Usuv6861F **GCTGTGTTTCTGATCTGTGT** / Usuv8956R **TGGCACTGCTCCATTGGTTC** (2095 bp)
- 8) Usuv8885F **TAAGAGGAAGGTCAACAGCA** / Usuv9994R **ACAACAGGAGCCACATCTGA** (1109 bp)
- 9) Usuv9643F\* **GAGAACGGAGAAGAAAGGGT** / Usuv10823R\* **ACCAGTTCGCATCACCGTCT** (1181 bp)

- 10) UsuvADF\* (10646) GAAAGCCCCTCAGAACCGTTTC / Usuv11066Rdeg AGATCCTGTGGTCTWGTGCC (420 bp) or
- 11) UsuvADF\* (10646) GAAAGCCCCTCAGAACCGTTTC / Usuv11014R\* AGATCCTGTGKTCTWSYYCMCCAYCAG (420 bp) or
- 12) Usuv10794F\*\* CAAGCGAACAGACGGTGATG / Usuv11027R\* GCGCTCTGTGCCTTGTGGTTGAT (233 bp)

Primers used for closing gap in reactions 4 and 7:

- 13) Usuv6901F GTGGCTGCCAATGAATACGGAA / Usuv7845R TGGGTGTCCCTCCAGTTTTGT (944 bp)
- 14) Usuv7769F AGAGGCCATCACTGAAGTCG / Usuv8908R CGTTGCTGTTGACCTTCCTC (1139 bp)
- 15) Usuv3167F TTGGAGTGATGGCGTTGTTG / Usuv4600R TTGCGTACTTGACAGTGAGC (1433 bp)

(\*) from Cadar *et al.* 2014

(\*\*) from Bakonyi *et al.* 2004

CLUSTAL multiple sequence alignment by MUSCLE (3.8)

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Senegal_ArD101291_1993  AGATGTTGGCCTGTGTGAGCTCTACTACTTAGTATTGTTTTTGGAGGATCGTGAGATTAA
Germania_V45_2011       AGTCGTTTCGTCTGCGTGAGCTCTACTACTTAGTATTGTTTTTGGAGGATCGTGAGATTAA
Germany_V382_2013      AGTCGTTTCGTCTGCGTGAGCTCTACTACTTAGTATTGTTTTTGGAGGATCGTGAGATTAA
Vienna_2001            AGTCGTTTCGTCTGCGTGAGCTCTACTACTTAGTATTGTTTTTGGAGGATCGTGAGATTAA
Bologna_2009           AGTCGTTTCGTCTGCGTGAGCTCTACTACTTAGTATTGTTTTTGGAGGATCGTGAGATTAA
Italia_2009            AGTCGTTTCGTCTGCGTGAGCTCTACTACTTAGTATTGTTTTTGGAGGATCGTGAGATTAA
Dakar_ArD19848_1974    AGATGTTGGCCTGTGTGAGCTCTACTACTTAGTATTGTTTTTGGAGGATCGTGAGATTAA
South_Africa_SAAR-1776_2001 AGTCGTTTCGTCTGCGTGAGCTCTACTACTTCATATTGGTTTTTGGAGGATCGTGAGATTAA
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South_Africa_SAAR-1776_2001 GGAAGAAACCGGGCCATCAATATGCTGAAACGCGGCATACCCCGCGTATTTCCCACTAGTG
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GCGCCTGCTTACAGCTTCAACTGCCTTGGTATGAGCAACAGAGACTTCCTTGAGGGAGTC  
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TCTGGTGCTACCTGGGTCGACGTGGTTCTGGAAGGCGACAGCTGCATAACCATCATGGCT  
TCTGGTGCCACCTGGGTCGACGTGGTTCTGGAAGGTGACAGCTGCATAACCATCATGGCT  
TCTGGTGCTACCTGGGTCGACGTGGTTCTGGAAGGTGACAGCTGCATAACCATCATGGCC  
TCTGGTGCTACCTGGGTTGACGTGGTTCTGGAAGGTGACAGCTGCATAACCATCATGGCC  
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TCTGGTGCCACCTGGGTCGACGTGGTTCTGGAAGGTGATAGCTGCATAACCATCATGGCT  
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GTCACTGACAGAGGCTGGGGCAATGGCTGTGGACTATTTGGCAAAGGAAGTATAGACACG  
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GTCACTGACAGGGGCTGGGGCAATGGCTGTGGACTATTTGGCAAAGGAAGTATAGACACG  
GTCACTGACAGGGGCTGGGGCAATGGCTGTGGACTATTTGGCAAAGGAAGTATAGACACG  
GTCACTGACAGAGGCTGGGGCAATGGCTGTGGACTATTTGGCAAAGGGGAGTATAGACACG  
GTTACTGACAGAGGCTGGGGCAATGGCTGTGGACTATTTGGCAAAGGAAGTATAGACACG  
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TGTGCCAACTTCACCTGCTCCCTGAAAGCGGTGGGCCGAATGATCCAACCGGAAAATGTT  
TGTGCCAACTTCACCTGCTCCCTGAAAGCGGTGGGCCGGATGATCCAACCGGAAAATGTT  
TGCGCCAACCTTCACCTGCTCCCTGAAAGCGACGGGCCGGATGATCCAACCGGAAAATGTT  
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TGTGCCAACTTCACCTGCTCCCTGAAAGCGATGGGCCGGATGATCCAACCGGAAAATGTT  
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TGTGCTAACTTCACCTGCTCCCTGAAAGCGATGGGCCGGATGATCCAACCGGAAAATGTT  
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TGTGCCAACTTCACCTGCTCCCTGAAAGCGGTGGGCCGAATGATCCAACCGGAAAATGTT  
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AAGTATGAAGTGGGAATCTTCATACATGGCTCCACTAGCTCTGACACTCATGGCAACTAT  
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AAGTATGAAGTGGGAATCTTCATACATGGTTCTACCAGCTCTGACACTCACGGCAACTAT  
AAGTATGAAGTGGGAATCTTCATACATGGTTCTACCAGCTCTGACACTCACGGCAACTAT  
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TCTTCACAACTAGGAGTATCACAGGCTGGGCGGTTTACCATCACTCCCAACTCCCCAGCC  
TCTTCACAACTAGGAGCATCACAGGCTGGGCGGTTTACCATCACTCCCAACTCCCCAGCC  
TCTTCACAACTAGGAGCATCGCAGGCTGGGCGGTTTACCATCACTCCCAACTCCCCAGCC  
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TCTTCACAACTAGGAGCATCACAGGCTGGGCGGTTTACCATCACTCCCAACTCCCCAGCC  
TCTTCACAACTAGGAGCATCACAGGCTGGGCGGTTTACCATCACTCCCAACTCCCCAGCC  
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TCTTCACAACTAGGAGCATCACAGGCTGGGCGGTTTACCATCACTCCCAACTCCCCAGCC  
TCTTCACAACTAGGAGCATCACAGGCTGGGCGGTTTACCATCACTCCCAACTCCCCAGCC  
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ATTACTGTGAAGATGGGTGACTATGGAGAAATATCAGTTGAGTGTGAACCAAGAAATGGG  
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ATCACTGTGAAGATGGGTGACTACGGAGAAATATCAGTTGAGTGTGAACCAAGAAACGGG  
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TTGAACACTGAGGCATACTACATCATGTTCAGTGGGCACCAAACACTTCCTTGTCCATAGA  
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TTGAACACCCGAGGCATACTACATCATGTTCAGTGGGCACCAAACACTTCCTTGTCCATAGA  
TTGAACACTGAGGCATACTACATCATGTTCAGTGGGCACCAAACACTTCCTTGTCCATAGA  
TTGAACACTGAGGCATACTACATCATGTTCAGTGGGCACCAAACACTTCCTTGTCCATAGA  
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GAATGGTTTAATGACTTGGCCCTCCCATGGACTTCACCAGCCAGCTCAAATTTGGAGAAAT  
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GAATGGTTCAATGACTTGGCCCTCCCATGGACTTCACCAGCTAGCTCAAATTTGGAGAAAT  
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GAATGGTTTAATGACTTGGCCCTCCCATGGACTTCACCAGCTAGCTCAAATTTGGAGAAAT  
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AGAGAAATACTACTAGAGTTTGAAGAACCCCATGCCACAAAGCAATCAGTTCGTGGCGCTT  
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GGTTCTCAGGAAGGTGCTTTGCACCAGGCCTTGGCAGGAGCTATTCAGTGTCTTTCTCG  
GGTTCCCAGGAAGGTGCTTTGCACCAGGCCTTGGCAGGAGCTGTTCCAGTGTCTTTCTCG  
GGTTCCCAGGAAGGTGCTTTGCACCAGGCCTTGGCAGGAGCTGTTCCAGTGTCTTTCTCG  
GGTTCCCAGGAAGGTGCTTTGCACCAGGCCTTGGCAGGAGCTGTTCCAGTGTCTTTCTCG  
GGTTCCCAGGAAGGTGCTTTGCACCAGGCCTTGGCAGGAGCTGTTCCAGTGTCTTTCTCG  
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GGTTCCCAGGAAGGTGCTTTGCACCAGGCCTTGGCAGGAGCTGTTCCAGTGTCTTTCTCG  
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GGTTCTCAGGAAGGTGCTTTGCACCAGGCCTTGGCAGGAGCTGTTCCAGTGTCTTTCTCG  
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AAAATTCCAATTTCCATCGTAGTATCACTTTCCGATCTCACTCCCATTGGTAGAATGGTT  
AAGATCCCAATTTCCATTGTGGCATCACTTTCCGATCTCACCCCATTTGGTAGAATGGTT  
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AAAATCCCAATTTCCATTGTGGCATCACTTTCCGATCTCACCCCATTTGGTAGAATGGTT  
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ACAGCAAACCCCTATGTGGCTTCATCCGAAGCCAACGCGAAAGTGCTGGTTGAGATGGAA  
ACAGCAAACCCCTTATGTGGCTTCATCCGAAGCCAACGCGAAAGTGCTGGTTGAGATGGAA  
ACAGCAAACCCCTTATGTGGCTTCATCCGAAGCCAACGCGAAAGTGCTGGTTGAGATGGAA  
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ACAGCAAACCCCTTATGTGGCTTCATCCGAAGCCAACGCGAAAGTGCTGGTTGAGATGGAA  
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CCACCATTTGGAGATTCATACATTGTGGTTGGAAGAGGGGACAAGCAGATAAACCATCAC  
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TCACCATTTGGAGATTCATACATTGTGGTTGGAAGAGGGGATAAGCAGATAAACCATCAC  
CCACCATTTGGAGATTCATATATTGTGGTTGGAAGAGGGGATAAGCAGATAAACCATCAC  
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TGGCACAAGGCAGGAAGCTCCATAGGAAAAGCGTTCATCACCACCATCAAAGGGGCACAG  
TGGCACAAGGCAGGAAGCTCCATTGGAAAAGCGTTCATCACTACTATCAAAGGAGCACAG  
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TGGCACAAGGCAGGAAGTTCATTGGAAAAGCGTTCATCACCACTATCAAAGGGGCACAG  
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CGTCTAGCTGCTCTAGGTGACACAGCTTGGGACTTTGGGTTCGGTTCGGAGGGATTTTCAAT  
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TCCGTAGGAAAGGCGGTACATCAGGTCTTTGGAGGAGCCTTCAGGACTCTCTTCGGCGGC  
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AGAGATCGATCCATCGCACTGGTGTAGTTAGCCACGGGAGGAGTGCTCCTCTTTCTCGCC  
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ACAAACGTCCATGCAGACTCGGGATGTGCGATAGACGTGGGAAGGAGAGAGTTGCGCTGT  
ACAAACGTCCATGCAGACTCGGGATGTGCGATAGACGTGGGAAGGAGAGAGTTGCGCTGT  
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ACAAACGTCCACGCAGACTCGGGATGTGCGATAGACGTAGGAAGGAGAGAGTTGCGCTGT  
ACAAGCGTCCATGCAGACTCGGGATGTGCGATAGACGTGGGAAGGAGAGAGTTGCGCTGT  
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GGACAAGGGATCTTCATCCACAATGATGTTGAAGCGTGGGTCGACCGGTACAAATTCATG  
GGACAAGGGATCTTCATCCACAATGATGTTGAAGCGTGGGTCGACCGGTACAAATTTATG  
GGACAAGGGATCTTCATCCACAATGATGTTGAAGCGTGGGTCGACCGGTACAAATTTATG  
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GGACAAGGGATCTTCATCCACAATGATGTTGAAGCGTGGGTCGACCGGTACAAATTTATG  
GGACAAGGGATCTTCATCCACAATGATGTTGAAGCGTGGGTCGACCGGTACAAATTTATG  
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CCTGAAACACCAAAACAACCTGGCAAAGGTCATCGAGCAAGCCCACGCGAAAGGAATATGT  
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CCTGAAACACCAAAACAGCTGGCAAAGGTCATCGAGCAAGCCCACGCGAAAGGAATATGT  
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GGACTGAGGTCTGTCTCACGTCTGGAACACGTGATGTGGGAGAACATCAGAGATGAACTC  
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AACACCCTCCTTAGAGAGAATGCAGTAGACCTAAGTGTCTGGTTGAGAAGCCAAAAGGA  
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AACACCCTCCTCAGAGAGAATGCAGTAGACCTAAGTGTCTGGTTGAGAAGCCAAAAGGA  
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ATGTACAAATCAGCACCCGAGAGACTGGCACTCACGTCTGAAGAGTTTGAGATTGGGTGG  
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AAGGCCTGGGGAAAGAGTTTGGTGTTCGCACCAGAACTGGCCAACCACACTTTTGTGGTT  
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AAAGCCTGGGGAAAGAGCTTGGTGTTCGCACCAGAACTGGCCAACCACACTTTTGTGGTT  
AAGGCCTGGGGAAAGAGCTTGGTGTTCGCACCAGAACTGGCCAACCACACTTTTGTGGTT  
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GATGGGCCTGAGACTAAAGAATGCCCGACGTAAAAAGAGCTTGGAACAGTCTTGAGATT  
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 GATGGGCCTGAGACCAAAGAATGCCCGACGTAAAAAGGGCTTGGAACAGCCTTGAGATT  
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GAAGACTTTGGATTTCGGCATCATGTCCACCAGGGTTTGGCTGAAAGTCAGAGAATACAAC  
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ACTACCGACTGCGACAGCTCAATAATTGGGACGGCTGTAAAGGAGACATAGCTGTGCAC  
 ACTACTGACTGCGACAGCTCAATAATTGGGACGGCTGTAAAGGAGACATAGCTGTGCAC  
 ACCACTGACTGCGACAGCTCAATAATTGGGACGGCTGTAAAGGAGACATAGCTGTGCAC  
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 ACCACTGACTGCGACAGCTCAATAATTGGGACGGCCGTTAAAGGAGACATAGCTGTGCAC  
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AGTGACCTCTCTTACTGGATTGAAAGCCACAAGAACACGACATGGAGGCTCGAGAGAGCT  
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AGTGACCTCTCCTACTGGATTGAAAGCCACAAGAACACGACATGGAGGCTCGAGAGGGCT  
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AGTGACCTCTCCTACTGGATTGAAAGCCACAAGAACACGACATGGAGGCTAGAGAGAGCT  
AGTGACCTCTCCTACTGGATTGAAAGCCATAAGAACACGACATGGAGGCTTGAGAGAGCT  
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GTCTTTGGAGAGATCAAATCATGCACGTGGCCTGAGACACACACTCTTTGGAGTGATGGT  
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GTCTTTGGAGAGATCAAGTCATGCACGTGGCCTGAGACACACACTCTTTGGAGTGATGGC  
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GTCTTTGGAGAGATCAAATCATGCACGTGGCCTGAGACACACACTCTTTGGAGTGATGGT  
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GTTGTTGAGAGTGATCTGGTTGTGCCAGTCACCCTCGCCGGGCCAAAAAGCAATCACAAC  
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CGGCGTGAAGGTTACAAAGTCCAGAGCCAAGGGCCGTGGGATGAAGAAGACATCATTCTC  
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GACTTTGACTATTGCCAGGTACCACCGTCACAATCACTGAAGCATGTGGGAAGAGAGGA  
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CCCTCCATAAGAACTACCCTAGCAGTGGTAGATTGGTCACAGACTGGTGCTGCCGGAGC  
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CCCTCCATAAGAACTACTACTAGCAGTGGTAGACTGGTCACAGACTGGTGCTGCCGGAGC  
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TGCACCCTTCCCCCTTTGAGATATAGGACAAAAAATGGATGTTGGTATGGAATGGAGATA  
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GCCTTCGCCGAAGCCAACAGCGGGGGTGACGTCGTCCATCTAGCTCTCATAGCCGTTTTT  
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GCCTTCGCCGAAGCCAACAGCGGGGGTGACGTCGTCCATCTAGCTCTCATAGCCGCCTTT  
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AAAATCCAACCAGGCTTTCTCACAATGACATTTCTTAGGGGAAAGTGGACGAATCAAGAG  
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AACATCCTGGCTGGCTCTGGGGGCAGCATTCTTTTCAGATGGCGGCCACTGATTTGAACTTC  
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TCTTTCCCAGGAATTCTCAATGCCACTGCCACGGCTTGGATGCTCCTGAGGGCTGCCACC  
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TCCCTTCCCAGGAATTCTCAATGCCACTGCCACAGCTTGGATGCTCCTGAGGGCTGCCACC  
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CAGCCATCTACTTCTGCCATCGTCATGCCTTTGCTCTGCCTATTGGCTCCTGGCATGAGA  
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CTGCTCTACCTGGACACGTATAGAATCACTCTCATCATCATCGGCATTTGCAGCCTGATA  
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GGGGAGCGCCGTAGAGCGGCCGCAAAGAAGAAAGGGGCGGTACTGCTAGGGTTAGCACTA  
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ACATCAACAGGGCAGTTTTCTGCGTCGGTTATGGCAGCTGGACTCATGGCATGCAATCCC  
ACATCAACAGGGCAGTTCTCTGCGTCAGTTATGGCGGCTGGGCTCATGGCATGCAATCCC  
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ACATCAACAGGGCAGTTCTCCTCGTCGGTTATGGCAGCTGGGCTCATGGCATGCAATCCC  
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AACAAAAAGCGGGGTGGCCGGCCACAGAAGTTTTGACAGCAGTTGGATTGATGTTTGCC  
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ATCGTGGGTGGTCTAGCTGAATTGGATGTTGATTCCATGTCCATTCCTTTTGTGTTGGCC  
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ATATGGGTCATCCGCATGACGGCACTAGGATTTCGCAGCCTGGACGCCATGGGCAATAATA  
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CCTGCCGAATAGGCTATTGGCTCACTGTCAAATACGCAAAAAGAGGAGGTGTCTTTTGG  
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GACACCCCGGCTCCAAGGACATACCCCAAGGGTGACACTTCACCAGGAGTATACCGTATC  
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GACACCCCGGCTCCAAGGACCTACCCCAAGGGTGACACTTCACCAGGAGTATACCGTATC  
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GACAGAAAGTGGGAATGGTCTCGATGATGTCCAGCTCATCATAGTGGCCCCCGGAAAGGCA  
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GCCATAAACATTCAAACCAAACCAGGTGTCTTCAAACGCCACAAGGGGAAATAGGAGCA  
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CCCCAGATCATCAAAGATGCCATTCAGCGCCGCCTCCGCACGGCTGTGTTGGCTCCGACC  
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CTAACCCACAGACTCATGTCCACCTCTAAGAGTTCCAAACTACAACCTCTTTGTGATGGAT  
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GAAGCCGACTTCACTGATCCAGCGAGCATAGCAGCACGAGGCTATATTGCCACAAAAGTT  
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GAAGCTCATTTCACTGACCCGCGGAGCATAGCAGCACGAGGCTACATTGCCACAAAAGTT  
GAAGCTCACTTCACTGATCCGCGGAGCATAGCAGCACGAGGCTATATTGCCACAAAAGTT  
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GAGTTGGGCGAAGCGGCAGCAATATTCATGACTGCAACTCCCCCTGGTACTCACGATCCG  
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CTCAACCGCAAGTCTTATGACACGGAGTATCCCAAATGCAAGAATGGAGACTGGGATTTT  
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CTCAACCGCAAGTCTTATGACACGGAGTATCCCAAATGCAAGAATGGAGACTGGGATTTT  
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GTGATAACAACAGACATCTCAGAGATGGGTGCGAACTTTGGGGCCAGCAGGGTCATTGAC  
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TGCCGAAAAGCGTAAAACCCACCATTCTGGAGGAAGGCCAAGGGAGAGTGATCTTGAGC  
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AACCCCTCACCAATCACTAGTGCTAGTGCCGCCAGAGGAGAGGAAGAGTAGGCAGAAAT  
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CCTAGTCAGATAGGTGATGAGTACCCTATGGAGGAAGCACAAGCGAAGATGACACGATT  
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GCAGCTCATTGGACTGAAGCAAAGATCATGTTAGATAACATCCACCTCCCAAATGGGCTT  
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ATTGCACAAATGTATGGGCCAGAAAGAGACAAAGCCTTTCACCATGGACGGGGAGTACCGG  
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GTTGCACAAATGTATGGGCCAGAAAGAGACAAAGCCTTTCACCATGGACGGGGAGTACCGT  
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CTGAGAGGAGAGGAGAGAAAGACCTTCCTGGAATTATTGAGGACTGCAGACCTGCCAGTG  
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TTGAGAGGAGAAGAGAGAAAGACCTTCCTGGAGTTGCTGAGGACTGCAGACCTGCCAGTG  
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TGGCTTGCCCTACAAAGTGGCTTCAAATGGTATACAGTACACCGACAGGAAGTGGTGT  
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GATGGACCCAGGTCAAACGTCATCTGGAAGACAACAATGAAGTCGAAATTGTCACCCGC  
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ACAGGTGAGCGCAAGATGCTGAAGCCACGCTGGCTAGATGCCAGGGTCTATTCCGACCAT  
ACAGGTGAACGCAAGATGCTGAAGCCACGCTGGTTAGATGCCAGGGTCTACGCTGACCAT  
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CAATCGCTCAAGTGGTTCAAGGACTTTCGCGGCTGGTAAGCGATCAGCAGTGGGATTCCTT  
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GAAGTCCTGGGGAGAATGCCTGAGCACTTCGCTGGCAAACCAGAGAGGCCTTTTGACACC  
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TCCCAAACGGACAACCAGCTTGCTGTGTTTCTGATCTGTGTCTTGCTGGTAGTGGGAGTG  
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TCCCAGACGGACAACCAGCTAGCTGTGTTTCTGATCTGTGTCTTGCTGGTGGTGGGAGTG  
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GTGGCTGCAAATGAATACGGAATGTTAGAGAGAACAAAAAGTGATCTTGGGAAAATGTTT  
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TCCAGCACACGTCAACCACAGAGTGCTCTGCCGCTACATTCATGAACGCTTTGGCATTG  
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GATTTGCGACCAGCAACAGCGTGGGCCCTATACGGAGGGAGCACAGTAGTTCTCACGCCC  
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TTGATCAAACATCTTGTAACATCAGAATACATCACAACATCTCTGGCATCAATAAGCGCT  
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CAGGCTGGGTCTGTTTCAACCTACCCCGCGGACTCCCCTTCACGGAGCTGGACTTGACA  
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GTGGTCTTGGTCTTTTTGGGATGCTGGGGCCAGGTGTCGTTAACAACCTCTGATTACTGCG  
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GCAGCTCTGGCTACCCTCCACTATGGCTACATGCTGCCTGGATGGCAGGCTGAAGCTTTG  
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AGGGCGGCTCAACGGAGAACAGCGGCCGGAATCATGAAAAATGCCGTGGTAGATGGCTTG  
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GTGGCTACGGATGTTCCCTGAATTGGAGAGAACCACCCCCCTTATGCAAAAAGAAGGTAGGC  
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CAGATTTTACTGATTGGGGTCAGCGCGGCGGCATTGTTAGTTAACCCGTGTGTTACAACA  
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GTTCGAGAAGCCGGCATCCTCATATCAGCGGCACTCCTGACTCTCTGGGACAACGGAGCC  
GTTCGGGAAGCCGGCATCCTCATATCAGCGGCACTCCTGACTCTCTGGGACAACGGAGCC  
GTCCGGGAAGCCGGCATCCTCATATCAGCGGCACTCCTGACTCTCTGGGACAACGGAGCC  
GTTCGGGAAGCCGGCATCCTCATATCAGCGGCACTCCTGACTCTCTGGGACAACGGAGCC  
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GTTCGGGAAGCCGGCATCCTCATATCAGCGGCACTCCTGACTCTCTGGGACAACGGAGCC  
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ATTGCAGTATGGAATTCCACCACCGCGACCGGACTTTGTACGTCATCCGTGGCAATTGG  
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CTGGCTGGAGCCTCTATAGCTTGGACTCTGATAAAGAATGCTGACAAACCGGCCTGCAAA  
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CGAGGAAGACCAGGAGGAAGAACAAGTGGAGCAGTGGAAAGAGAAGCTGAATGGGCTC  
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CGAGGAAGACCAGGAGGAAGGACACTGGGTGAGCAATGGAAGGAGAAGCTAAACGGGCTC  
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CGCACTCTGAGGATTTTGGAAATGGTCTCTGATTGGCTGCAGAGAGGACCAAGAGAGTTC  
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TGTATCAAGGTTCTCTGCCCATACATGCCACGTGTCATGGAGCGCTTAGAAGTTCTACAA  
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CGGAGGTATGGAGGAGGATTGGTTCGAGTCCCTCTTTCCAGAAATCCAACCATGAGATG  
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TACTGGGTCAGTGGAGCTGCTGGCAACATTGTTACGCAGTGAACATGACGAGTCAAGTG  
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CTCATAGGGCGAATGGAGAAGAGAACATGGCATGGACCAAATACGAGGAGGATGTTAAC  
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CTTGGAAGTGGAAACAAGAGCTGTGCGGAAGCCCCAGCCACATGCCAATCAGGAGAAGATT  
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AAAGCCAGGATTCAAAGATTGAAAGAGGAGTATGCAGCCACATGGCACCATGACAAGGAC  
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CACCCATATCGGACTTGGACCTACCACGGGAGCTATGAAGTCAAACCGACCGGTTTCAGCA  
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AGCTCCTTGGTCAACGGAGTCGTCCGCCTAATGAGCAAGCCCTGGGATGCAATTCTCAAT  
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GTGACCACATGGCGATGACTGACACCCTCCGTTTGGGCAGCAGAGGGTCTTCAAAGAG  
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AAGGTTGATACCAAGGCCCCAGAACCCCTTCTGGAGTTAAAGAGGTGATGGATGAGACC  
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ACCAATTGGCTGTGGGCTTTTCTCGCACGAGAAAAGAAGCCAAGGTTGTGCACCAGGGAA  
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GAGTTCAAGAGGAAGGTCAACAGCAACGCTGCTTTGGGTGCCATGTTTGAAGAGCAGAAC  
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CAATGGAGCAGTGCTAGGGAGGCTGTAGAGGACCCTCGGTTCTGGGAAATGGTGGATGAA  
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Dakar\_ArD19848\_1974  
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GAAGGTGAGCACAGACAACCTGGCCAGAGCAATCATTGAGCTGACCTACAAACACAAGGTG  
GAAGGTGAGCACAGACAACCTGGCTAGAGCAATCATTGAGCTGACCTACAAACACAAGGTG  
GAAGGTGAGCACAGACAACCTGGCTAGAGCAATCATTGAGCTGACCTACAAACACAAGGTG  
GAAGGTGAGCACAGACAACCTAGCCAGAGCAATCATTGAGCTGACCTACAAACACAAGGTG  
GAAGGTGAGCACAGACAACCTAGCAAGAGCAATCATTGAGCTGACCTACAAACACAAGGTG  
GAAGGTGAGCACAGACAACCTAGCCAGAGCAATCATTGAGCTGACCTACAAACACAAGGTG  
GAAGGTGAGCACAGACAACCTAGCCAGAGCAATCATTGAGCTGACCTACAAACACAAGGTG  
GAAGGTGAGCACAGACAACCTAGCCAGAGCAATCATTGAGCTGACCTACAAACACAAGGTG  
GAAGGTGAGCACAGACAACCTAGCCAGAGCAATCATTGAGCTGACCTACAAACACAAGGTG  
GAAGGTGAGCACAGACAACCTGGCCAGAGCAATCATTGAGCTGACCTACAAACACAAGGTG  
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Spain\_MB119/06\_2006,  
Dakar\_ArD192495,  
Senegal\_ArD101291\_1993  
Germania\_V45\_2011  
Germany\_V382\_2013  
Vienna\_2001  
Bologna\_2009  
Italia\_2009  
Dakar\_ArD19848\_1974  
South\_Africa\_SAAR-1776\_2001

GTGAAAGTTATGCGACCTGGCACAGATGGGAAGACCGTCATGGATGTGATCTCCCGAGAA  
GTGAAAGTTATGCGACCTGGCACAGATGGGAAGACCGTCATGGATGTGATTTCCCGAGAA  
GTGAAAGTTATGCGACCTGGCACAGATGGGAAGACCGTCATGGATGTGATTTCCCGAGAA  
GTGAAAGTTATGCGACCTGGCACAGATGGGAAGACCGTCATGGATGTGATTTCCCGAGAA  
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GTGAAAGTTATGCGACCTGGCACAGATGGGAAGACCGTCATGGATGTGATTTCCCGAGAA  
GTGAAAGTTATGCGACCTGGCACAGATGGGAAGACCGTCATGGATGTGATTTCCCGAGAA  
GTGAAAGTTATGCGACCTGGCACAGATGGGAAGACCGTCATGGATGTGATTTCCCGAGAA  
GTGAAAGTTATGCGACCTGGCACTGATGGGAAGACCGTCATGGATGTGATTTCCCGAGAA  
GTGAAAGTTATGCGACCTGGCACAGATGGGAAGACCGTCATGGATGTGATTTCCCGAGAA  
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Spain\_MB119/06\_2006,  
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Senegal\_ArD101291\_1993  
Germania\_V45\_2011  
Germany\_V382\_2013  
Vienna\_2001  
Bologna\_2009  
Italia\_2009  
Dakar\_ArD19848\_1974  
South\_Africa\_SAAR-1776\_2001

GACCAGAGAGGAAGTGGACAGGTTGTGACCTATGCTCTCAACACATTCACCAACATTGCT  
GATCAGAGAGGAAGTGGACAGGTTGTGACCTATGCTCTCAACACATTCACCAACATTGCC  
GATCAGAGAGGAAGTGGACAGGTTGTGACCTATGCTCTCAACACATTCACCAACATCGCC  
GATCAGAGAGGAAGTGGACAGGTTGTGACCTATGCTCTCAACACATTCACCAACATTGCC  
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GATCAGAGAGGAAGTGGACAGGTTGTGACCTATGCTCTCAACACATTCACCAACATTGCC  
GATCAGAGAGGAAGTGGACAGGTTGTGACCTATGCTCTCAACACATTCACCAACATTGCC  
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GATCAGAGAGGAAGTGGACAGGTTGTGACCTATGCTCTCAACACATTCACCAACATTGCC  
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Spain\_MB119/06\_2006,  
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Italia\_2009  
Dakar\_ArD19848\_1974  
South\_Africa\_SAAR-1776\_2001

GTCCAGCTCATCAGACTGATGGAAGCTGAAGGAGTGATTGGGCAGGAACATTTGGAAAGT  
GTCCAGCTTATCAGACTGATGGAAGCTGAAGGAGTGATAGGGCAGGAACATCTGGAAAGT  
GTCCAGCTTATCAGACTGATGGAAGCTGAAGGAGTGATTGGGCAGGAACATCTGGAAAGT  
GTCCAGCTCATTAGACTGATGGAAGCTGAAGGAGTGATTGGGCAGGAACATCTGGAAAGT  
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GTCCAGCTCATTAGACTGATGGAAGCTGAAGGAGTGATTGGGCAGGAACATCTGGAAAGT  
GTCCAGCTCATTAGACTGATGGAAGCTGAAGGAGTGATTGGGCAGGAACATCTGGAAAGT  
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Spain\_MB119/06\_2006,  
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Germany\_V382\_2013  
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Italia\_2009  
Dakar\_ArD19848\_1974  
South\_Africa\_SAAR-1776\_2001

CTTCCCCGAAAACCAAATACGCTGTGAGAACCCTGGCTCTTTGAGAACGGAGAAGAAAGA  
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CTTCCCCGAAAACCAAATACGCTGTGAGAACCCTGGCTCTTTGAGAACGGAGAAGAAAGG  
CTTCCCCGAAAACCAAATACGCTGTGAGAACCCTGGCTCTTTGAGAACGGAGAAGAAAGG  
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CTTCCCCGAAAACCAAATACGCTGTGAGAACCCTGGCTCTTTGAGAACGGAGAAGAAAGG  
CTTCCCCGAAAACCAAATACGCTGTGAGAACCCTGGCTCTTTGAGAACGGAGAAGAAAGG  
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Spain\_MB119/06\_2006,  
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GTAACCCGTATGGCTGTGAGTGGAGATGATTGCGTTGTCAAGCCCCCTGGATGATCGGTTT  
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GTGACCCGCATGGCTGTGAGTGGAGATGATTGTGTTGTCAAGCCCCCTGGATGATCGGTTT  
GTAACCCGCATGGCTGTGAGTGGAGATGATTGTGTTGTCAAGCCCCCTGGATGATCGGTTT  
GTAACCCGCATGGCTGTGAGTGGAGATGATTGTGTTGTCAAGCCCCCTGGATGATCGGTTT  
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GCCAATGCCCTGCACTTTCTCAATTCGATGTCTAAGGTCAGAAAAGACGTGCCAGAGTGG  
GCCAATGCCCTGCACTTTCTCAATTCGATGTCCAAGGTCAGAAAAGATGTGCCAGAGTGG  
GCCAATGCCCTGCACTTTCTCAATTCGATGTCCAAGGTCAGAAAAGACGTGCCAGAGTGG  
GCCAATGCCCTGCACTTTCTCAATTCGATGTCCAAGGTCAGAAAAGACGTGCCAGAGTGG  
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GCCAATGCCCTGCACTTTCTCAATTCGATGTCCAAGGTCAGAAAAGACGTGCCAGAGTGG  
GCCAATGCCCTGCACTTTCTCAATTCGATGTCCAAGGTCAGAAAAGACGTGCCAGAGTGG  
GCCAATGCCCTGCACTTTCTCAATTCGATGTCTAAGGTCAGAAAAGACGTGCCAGAGTGG  
GCCAATGCCCTGCACTTTCTCAATTCGATGTCTAAGGTCAGAAAAGACGTGCCAGAGTGG  
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Italia\_2009  
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AAACCCTCCTCGGGATGGCAGCATTGGCAGCAAGTGCCTTTCTGCTCAAACCACTTTTCAG  
AAACCCTCCTCGGGATGGCAGCATTGGCAGCAAGTGCCTTTCTGCTCAAACCACTTCCAG  
AAACCCTCCTCGGGATGGCAGCATTGGCAGCAAGTGCCTTTCTGCTCAAACCACTTTTCAG  
AAACCCTCCTCGGGATGGCAGCATTGGCAGCAAGTGCCTTTCTGCTCAAACCACTTTTCAG  
AAACCCTCCTCGGGATGGCAGCATTGGCAGCAAGTGCCTTTCTGCTCAAACCACTTTTCAG  
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AAACCCTCCTCGGGATGGCAGCATTGGCAGCAAGTGCCTTTCTGCTCAAACCACTTTTCAG  
AAACCCTCCTCGGGATGGCAGCATTGGCAGCAAGTGCCTTTCTGCTCAAACCACTTTTCAG  
AAACCCTCCTCGGGATGGCAGCATTGGCAGCAAGTGCCTTTCTGCTCAAACCACTTTTCAG  
AAACCCTCCTCGGGATGGCAGCATTGGCAGCAAGTGCCTTTCTGCTCAAACCACTTTTCAG  
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Italia\_2009  
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GAATTGATCATGAAGGACGGAAGGACTTTGGTGGTTCCCTGCCGAGGCCAGGATGAACTC  
GAATTGATCATGAAGGACGGAAGAACTTTGGTGGTTCCCTGCCGGGGTCAGGATGAACTC  
GAATTGATCATGAAGGACGGAAGGACTTTGGTGGTTCCCTGCCGGGGTCAGGATGAACTC  
GAATTGATCATGAAGGACGGAAGGACTTTGGTGGTTCCCTGCCGGGGTCAGGATGAACTC  
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GAATTGATCATGAAGGACGGAAGGACTTTGGTGGTTCCCTGCCGGGGTCAGGATGAACTC  
GAATTGATCATGAAGGACGGAAGGACTTTGGTGGTTCCCTGCCGGGGTCAGGATGAACTC  
GAATTGATCATGAAGGACGGAAGGACTTTGGTGGTTCCCTGCCGAGGTCAGGATGAACTC  
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Italia\_2009  
Dakar\_ArD19848\_1974  
South\_Africa\_SAAR-1776\_2001

TGGTCAGTGCATGCCACAGGTGAATGGATGACAACCTGATGACATGTTGGAAGTGTGGAAC  
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TGGTCAGTGCATGCCACAGGTGAATGGATGACAACCTGATGACATGTTGGAAGTGTGGAAC  
TGGTCAGTGCATGCCACAGGTGAATGGATGACAACCTGATGACATGTTGGAAGTGTGGAAC  
TGGTCAGTGCATGCCACAGGTGAATGGATGACAACCTGATGACATGTTGGAAGTGTGGAAC  
TGGTCAGTGCATGCCACAGGTGAATGGATGACAACCTGATGACATGTTGGAAGTGTGGAAC  
TGGTCAGTGCATGCCACAGGTGAATGGATGACAACCTGATGACATGTTGGAAGTGTGGAAC  
TGGTCAGTGCATGCCACAGGTGAATGGATGACAACCTGATGACATGTTGGAAGTGTGGAAC  
TGGTCAGTGCATGCCACAGGTGAATGGATGACAACCTGATGACATGTTGGAAGTGTGGAAC  
TGGTCAGTGCATGCCACAGGTGAATGGATGACAACCTGATGACATGTTGGAAGTGTGGAAC  
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Italia\_2009  
Dakar\_ArD19848\_1974  
South\_Africa\_SAAR-1776\_2001

AAAGTGTGGATTCAAGACAATGAATGGATGCTGGACAAGACCCAGTTCAAAGCTGGACA  
AAAGTGTGGATTCAAGACAATGAATGGATGCTGGACAAGACCCAGTTCAAAGCTGGACA  
AAAGTGTGGATTCAAGACAACGAATGGATGCTGGACAAGACCCAGTTCAAAGCTGGACA  
AAAGTGTGGATTCAAGACAACGAATGGATGCTGGACAAGACCCAGTTCAAAGCTGGACA  
AAAGTGTGGATTCAAGACAACGAATGGATGCTGGACAAGACCCAGTTCAAAGCTGGACA  
AAAGTGTGGATTCAAGACAACGAATGGATGCTGGACAAGACCCAGTTCAAAGCTGGACA  
AAAGTGTGGATTCAAGACAATGAATGGATGCTGGACAAGACCCAGTTCAAAGCTGGACA  
AAAGTGTGGATTCAAGACAATGAATGGATGCTGGACAAGACCCAGTTCAAAGCTGGACA  
AAAGTGTGGATTCAAGACAATGAATGGATGCTGGACAAGACCCAGTTCAAAGCTGGACA  
AAAGTGTGGATTCAAGACAATGAATGGATGCTGGACAAAACCCAGTTCAAAGCTGGACA  
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Spain\_MB119/06\_2006,  
Dakar\_ArD192495,  
Senegal\_ArD101291\_1993  
Germania\_V45\_2011  
Germany\_V382\_2013  
Vienna\_2001  
Bologna\_2009  
Italia\_2009  
Dakar\_ArD19848\_1974  
South\_Africa\_SAAR-1776\_2001

GACATCCCTTACACCGGGAAGCGGGAAGACATATGGTGTGGAAGCCTGATAGGCACGCGA  
GACATCCCTTACACCGGGAAGCGGGAAGACATATGGTGTGGAAGCCTGATAGGCACGCGA  
GACATCCCTTACACCGGGAAGCGGGAAGACATATGGTGTGGAAGCCTGATAGGCACGCGA  
GACATCCCTTACACCGGGAAGCGGGAAGACATATGGTGTGGAAGCCTGATAGGCACGCGA  
GACATCCCTTACACCGGGAAGCGGGAAGACATATGGTGTGGAAGCCTGATAGGCACGCGA  
GACATCCCTTACACCGGGAAGCGGGAAGACATATGGTGTGGAAGCCTGATAGGCACGCGA  
GACATCCCTTACACCGGGAAGCGGGAAGACATATGGTGTGGAAGCCTGATAGGCACGCGA  
GACATCCCTTACACCGGGAAGCGGGAAGACATATGGTGTGGAAGCCTGATAGGCACGCGA  
GACATCCCTTACACCGGGAAGCGGGAAGACATATGGTGTGGAAGCCTGATAGGCACGCGA  
GACATCCCTTACACCGGGAAGCGGGAAGACATATGGTGTGGAAGCCTGATAGGCACGCGA  
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Spain\_MB119/06\_2006,  
Dakar\_ArD192495,  
Senegal\_ArD101291\_1993  
Germania\_V45\_2011  
Germany\_V382\_2013  
Vienna\_2001  
Bologna\_2009  
Italia\_2009  
Dakar\_ArD19848\_1974  
South\_Africa\_SAAR-1776\_2001

TGTAATTTAGTTGTAAGTATTTGATTGTGTAGCTTTACTTAGCATCATTTTAGGATAATA  
TGTAATTTAGTTGTAAATATTTGATTGTGTAGCTTTATCTAGCATTATTTTAGGATAGTA  
TGTAATTTAGTTGTAAATATTTGATTGCGTAGCTCTATTTAGTGTGTTTTAGGATAGTA  
TGTAATTTAGTTGTAAATATTTGATTGTGTAGCTTTATTTAGCATTGTTTTAGGATAGTA  
TGTAATTTAGTTGTAAATATTTGATTGTGTAGCCTTATTTAGCATTGTTTTAGGATAGTA  
TGTAATTTAGTTGTAAATATTTGATTGTGTAGCTTTATTTAGCATTGTTTTAGGATAGTA  
TGTAATTTAGTTGTAAATATTTGATTGTGTAGCTTTATTTAGCATTGTTTTAGGATAGTA  
TGTAATTTAGTTGTAAATATTTGATTGTGTAGCTTTATTTAGTATCATTTTAGGATAGTA  
TGTAATTTAGTTGTAAGTATTTGATTGTGTAGCTTTATTTAGTATCATTTTAGGATAATA  
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Spain\_MB119/06\_2006,  
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Germania\_V45\_2011  
Germany\_V382\_2013  
Vienna\_2001  
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Italia\_2009  
Dakar\_ArD19848\_1974  
South\_Africa\_SAAR-1776\_2001

GAAGTTAAGTTTTGATTTAGTTATTTTATTTAATTGGATTTGATAGTCAGGCCAGGGCAA  
GAAGTTAAGTTTTGTTTAGTTATTTTATTTAATTGAATTTGATAGTCAGGCCAGGGCAA  
GAAGTTAAGTTTTATTTAGTTATTTTATTTAATTGAATTTGATAGTCAGGCCAGGGCAA  
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GAAGTTAAGTTTTATTTAGTTATTTTATTTAATTGAATTTGATAGTCAGGCCAGGGCAA  
GAAGTTGAGTTTGTATTTAGTTATTTTATTTAATTGAATTTGATAGTCAGGCCAGGGTAA  
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Spain\_MB119/06\_2006,  
Dakar\_ArD192495,  
Senegal\_ArD101291\_1993  
Germania\_V45\_2011  
Germany\_V382\_2013  
Vienna\_2001  
Bologna\_2009  
Italia\_2009  
Dakar\_ArD19848\_1974  
South\_Africa\_SAAR-1776\_2001

CCTGCCACCGGAAGTTGAGTAGACGGTGCCTGCGACTCAACCCAGGCGGACTGGGT  
CCTGCCACCGGAAGTTGAGTAGACGGTGCCTGCGACTCAACCCAGGCGGACTGGGT  
CCTGCCACCGGAAGTTGAGTAGACGGTGCCTGCGACTCAACCCAGGCGGACTGGGT  
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CCTGCCACCGGAAGTTGAGTAGACGGTGCCTGCGACTCAACCCAGGCGGACTGGGT  
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Germany\_V382\_2013  
Vienna\_2001  
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Italia\_2009  
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South\_Africa\_SAAR-1776\_2001

TAACAAAGCTGGCCGCTGATGATAGGAAAGCCCCCTCAGAACCGTCTCGGAGAGGGACCCT  
TAACAAAGCTGACCGCTGATGATAGGAAAGCCCCCTCAGGACCGTTTCGGAGAGGGACCCT  
TAACAAAGCTGACCGCTGATGATAGGAAAGCCCCCTCAGAACCGTTCGGAGAGGGACCCT  
TAGCAAAGCTGACCGCTGATGATGGGAAAGCCCCCTCAGAACCGTTCGGAGAGGGACCCT  
TAGCAAAGCTGACCGCTGATGATGGGAAAGCCCCCTCAGAACCGTTCGGAGAGGGACCCT  
TAACAAAGCTGACCGCTGATGATGGGAAAGCCCCCTCAGAACCGTTCGGAGAGGGACCCT  
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TAACAAAGCTGACCGCTGATGATGGGAAAGCCCCCTCAGAACCGTTCGGAGAGGGACCCT  
TATCAAAGCTGGCCGCTGATGATAGGAAAGCCCCCTCAGAACCGTTCGGAGAGGGACCCT  
TAACAAAGCTGGCCGCTGATGATAGGAAAGCCCCCTCAGAACCGTTCGGAGAGGGACCCT  
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Germania\_V45\_2011  
Germany\_V382\_2013  
Vienna\_2001  
Bologna\_2009  
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South\_Africa\_SAAR-1776\_2001

GCCTATTGGAAGCGTTCAGCCCGTGTGTCAGGCCGCAAAGCGCCACTTCGCCAAGGAGTGCA  
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GCCTATTGGAAGCGTCCAGCCCGTGTGTCAGGCCGCAAAGCGCCACTTCGCCAAGGAGTGCA  
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GCCTATTGGAAGCGTCCAGCCCGTGTGTCAGGCCGCAAAGCGCCACTTCGCCAAGGAGTGCA  
GCCTATTGGAAGCGTCCAGCCCGTGTGTCAGGCCGCAAAGCGCCACTTCGCCAAGGAGTGCA  
GCTTATTGGAAGCGTCCAGCCCGTGTGTCAGGCCGCAAAGCGCCACTTCGCCAAGGAGTGCA  
GCTTATTGGAAGCGTCCAGCCCGTGTGTCAGGCCGCAAAGCGCCACTTCGCCAAGGAGTGCA  
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Germania\_V45\_2011  
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Italia\_2009  
Dakar\_ArD19848\_1974  
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GCCTGTATGGCCCCAGGAGGACTGGGTTACCAAAGCCGAAAGGCCCCACGGCCCAAGCG  
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GCCTGTATGGCCCCAGGAGGACTGGGTTACCAAAGCCGAAAGGCCCCACGGCCCAAGCG  
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Spain\_MB119/06\_2006, AACAGACGGTGATGCGAACTGTTTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGA  
Dakar\_ArD192495, AACAGACGGTGATGCGAACTG-TTCG-----  
Senegal\_ArD101291\_1993 AACAGACGGTGATGCGAACTG-TTCGTGGAAGGACTAG-----  
Germania\_V45\_2011 AACAGACGGTGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGA  
Germany\_V382\_2013 AACAGACGGTGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGA  
Vienna\_2001 AACAGACGGTGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGA  
Bologna\_2009 AACAGACGGTGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGA  
Italia\_2009 AACAGACGGTGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGA  
Dakar\_ArD19848\_1974 AACAGACGGTGATGCGAACTG-TTCGTGGAAGGACTAG-----  
South\_Africa\_SAAR-1776\_2001 AACAGACGGTGATGCGAACTG-TTCGTGGAAGGACTAGAGGTTAGAGGAGACCCCGTGGA  
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Spain\_MB119/06\_2006, ACTTAGGTGCGGCCCAAGCCGTTTCCGAAGCTGTAGGAACGGTGGAAGGACTAGAGGTTA  
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Senegal\_ArD101291\_1993 -----  
Germania\_V45\_2011 ACTTAGGTGCGGCCCAAGCCGTTTCCGAAGCTGTAGGAACGGTGGAAGGACTAGAGGTTA  
Germany\_V382\_2013 ACTTAGGTGCGGCCCAAGCCGTTTCCGAAGCTGTAGGAACGGTGGAAGGACTAGAGGTTA  
Vienna\_2001 ACTTAGGTGCGGCCCAAGCCGTTTCCGAAGCTGTAGGAACGGTGGAAGGACTAGAGGTTA  
Bologna\_2009 ACTTAGGTGCGGCCCAAGCCGTTTCCGAAGCTGTAGGAACGGTGGAAGGACTAGAGGTTA  
Italia\_2009 ACTTAGGTGCGGCCCAAGCCGTTTCCGAAGCTGTAGGAACGGTGGAAGGACTAGAGGTTA  
Dakar\_ArD19848\_1974 -----  
South\_Africa\_SAAR-1776\_2001 ACTTAGGTGCGGCCCAAGCCGTTTCCGAAGCTGTAGGAGCGGTGGAAGGACTAGAGGTTA

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Senegal\_ArD101291\_1993 -----  
Germania\_V45\_2011 GAGGAGACCCCGCATCATAAGCATC-AAAAAACAGCATATTGACACCTGGGAATTAGACT  
Germany\_V382\_2013 GAGGAGACCCCGCATCATAAGCATC-AAAAAACAGCATATTGACACCTGGGAATTAGACT  
Vienna\_2001 GAGGAGACCCCGCATCATAAGCATCAAAAAAACAGCATATTGACACCTGGGAATTAGACT  
Bologna\_2009 GAGGAGACCCCGCATCATAAGCATC-AAAAAACAGCATATTGACACCTGGGAATTAGACT  
Italia\_2009 GAGGAGACCCCGCATCATAAGCATC-AAAAAACAGCATATTGACACCTGGGAATTAGACT  
Dakar\_ArD19848\_1974 -----  
South\_Africa\_SAAR-1776\_2001 GAGGAGACCCCGCATCATAAGCATC--AAAACAGCATATTGACACCTGGGAATTAGACT



Spain_MB119/06_2006,	AGGAGATCTTCTGCTCTATTCCAACATCAACCACAAGGCACAGAGCGCCGAAAATTGTGG
Dakar_ArD192495,	-----
Senegal_ArD101291_1993	-----
Germania_V45_2011	AGGAGATCTTCTGCTCTATTCCAACATCAACCACAAGGCACAGAGCGCCGAAAATTGTGG
Germany_V382_2013	AGGAGATCTTTTGCTCTATTCCAACATCAACCACAAGGCACAGAGCGCCGAAAATTGTGG
Vienna_2001	AGGAGATCTTCTGCTCTATTCCAACATCAACCACAAGGCACAGAGCGCCGAAAATTGTGG
Bologna_2009	AGGAGATCTTCTGCTCTATTCCAACATCAACCACAAGGCACAGAGCGCCGAAAATTGTGG
Italia_2009	AGGAGATCTTCTGCTCTATTCCAACATCAACCACAAGGCACAGAGCGCCGAAAATTGTGG
Dakar_ArD19848_1974	-----
South_Africa_SAAR-1776_2001	AGGAGATCTTCTGCTCTATTCCAACATCAACCACAAGGCACAGAGCGCCGAAAATTGTGG

Spain_MB119/06_2006,	CTGGTGGGGAAGACTAGACCACAGGATCT
Dakar_ArD192495,	-----
Senegal_ArD101291_1993	-----
Germania_V45_2011	CTGATGGGGGGCTAGACCACAGGATCT
Germany_V382_2013	CTGATGGGGAACAAGACAACAGGATCT
Vienna_2001	CTGGTGGGGAAGACTAGACCACAGGATCT
Bologna_2009	CTGATGGGGAACAAGACCACAGGATCT
Italia_2009	TTGGTGGGGAAGACTAGACCACAGGATCT
Dakar_ArD19848_1974	-----
South_Africa_SAAR-1776_2001	CTGGTGGGGAAGACTAGACCACAGGATCT

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 PhD student Roberto Bechere

Technical Appendix (Table S2)

*Usutu* virus, reference strain “Vienna2001” complete genome sequence (AY453411)

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121 ggaagaaacc gggccatcaa tatgctgaaa cgcggcatac cccgcgtatt cccactagtg
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361 ggaacaatga tcaacgtggt taacaatcgg ggcacaaaaa agaagagagg caacaatgga
421 ccaggactag tgatgatcat aacactcatg acggttgttt caatggtttc ctctttaaag
481 ctttccaact tccaggggaa agtcatgatg accatcaacg cgactgatat ggcggatgtc
541 attggtgttc ccacgcaaca tgggaaaaac cagtgtctgga ttagagccat ggatgtcggg
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661 gaagacattg actgttgggtg tgacaacaaa cccatgtatg ttcactatgg aagggtcaca
721 agaaccagac actcgaagcg gagtcggcgg tcgatcgcag tgcagacgca cggggagagt
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961 gcgctgctt atagcttcaa ctgccttggt atgagcaaca gagacttctc tgagggagtc
1021 tctggtgcta cctgggttga cgtggttttg gaaggtgaca gctgcataac catcatggcc
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1141 gtgagaagct actgctatct agccactgtc tcagatgttt caactgtctc caaatgtcca
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1381 aagtatgaag tgggaatctt catacatggt tctaccagct ctgacactca cggcaactat
1441 tcttcacaac taggagcatc acaggctggg cggtttacca tcaactccaa ctccccagcc
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2101 ccaccatttg gagattcata cattgtggtt ggaagagggg ataagcagat aaaccatcac
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2221 cgtctagtag ccttaggcga cacagcgtgg gactttgggt cggtcggagg gattttcaat
2281 tctgtaggaa aggggtgaca tcaggctctt ggaggagcct tcagaactct cttcgggtggc
2341 atgtcctgga tcaaccaggg tctaattggga gctctgcttc tatggatggg ggtgaatgcg
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2581 cctgaaacac caaaacagct ggcaaaggtc atcgagcaag cccacgcgaa aggaatatgt
2641 ggattgaggt ccgtctcacg tctggaacac gtgatgtggg agaacatcag agatgaactc
    
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Roberto Bechere

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2701 aacaccctcc tcagagagaa tgcagtagat ctaagtgtcg tggttgagaa gccaaaagga  
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2881 gacgggcccg agaccaaaaga atgtcccgat gcaaaaagag cttggaacag ctttgagatt  
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3121 gtctttggag agatcaaate atgcacgtgg cctgagacac acactctt **ttg gagtgatggc**  
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9481 gatcagagag gaagtggaca ggttgtgacc tatgctctca acacattcac caacattgce

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9541 gtccagctta ttagactgat ggaagctgaa ggagtgattg ggcaggaaca tctggaaagt  
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 10921 aggagacccc gcatcataag catcaaaaaa acagcatatt gacacctggg aattagacta  
 10981 ggagatcttc tgctctattc caacatcaac cacaaggcac agagcgc cga aaattgtggc  
 11041 tggtggggaa cttagaccaca ggatct

Primers used for the full-length amplification of *Usutu* virus

- 1) Usuv F1deg / Usuv327R
- 2) Usuv232F / Usuv1246R
- 3) Usuv1124F / Usuv2353R
- 4) Usuv2295F / Usuv4693R
- 5) Usuv4578F / Usuv5509R
- 6) Usuv5443F / Usuv6935R
- 7) Usuv6861F / Usuv8956R
- 8) Usuv8885F / Usuv9994R
- 9) Usuv9643F\* / Usuv10823R\*

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- 10) UsuvADF (10646) F\* / Usuv11066Rdeg or UsuvADF (10646) F\* / Usuv11014R\*
- 11) Usuv10794F\*\* / Usuv11027R\*
- 12) Usuv6901F / Usuv7845R
- 13) Usuv7769F / Usuv8908R
- 14) Usuv3167F / Usuv4600R

(\*) from Cadar *et al* 2014

(\*\*) from Bakonyi *et al* 2004