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Short Communication

Characterization of the Sandfly fever Naples species complex and description of a new Karimabad species complex (genus *Phlebovirus*, family *Bunyaviridae*)

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Genomic and antigenic characterization of members of the Sandfly fever Naples virus (SFNV) complex reveals the presence of five clades that differ in their geographical distribution. Saint Floris and Gordil viruses, both found in Africa, form one clade; Punique, Granada and Massilia viruses, all isolated in the western Mediterranean, constitute a second; Toscana virus, a third; SFNV isolates from Italy, Cyprus, Egypt and India form a fourth; while Tehran virus and a Serbian isolate Yu 8/76, represent a fifth. Interestingly, this last clade appears not to express the second non-structural protein ORF. Karimabad virus, previously classified as a member of the SFNV complex, and Gabek Forest virus are distinct and form a new species complex (named Karimabad) in the *Phlebovirus* genus. In contrast with the high reassortment frequency observed in some South American phleboviruses, the only virus of the SFNV complex with evidence of reassortment was Granada virus.

The more than 350 named RNA viruses included in the family *Bunyaviridae* are divided into five genera: *Orthobunyavirus*, *Nairovirus*, *Hantavirus*, *Phlebovirus* and *Tospovirus* (Nichol *et al.*, 2005). Bunyavirus genomes range from 11–19 kilobases in length and comprise three unique molecules of negative or ambisense ssRNA, designated L (large), M (medium) and S (small). Viruses in each genus share similar segment and structural protein sizes and have characteristic terminal sequences at the 3′ and 5′ ends of each segment. Similar to other segmented virus families, genetic reassortment has been demonstrated among related bunyaviruses both *in vitro* and *in vivo* (Henderson *et al.*, 1995; Li *et al.*, 1995; Pringle *et al.*, 1984; Rodriguez *et al.*, 1998).

Except for the tospoviruses, which only infect plants, human pathogens are found in each of the other four genera. At present, the genus *Phlebovirus* comprises approximately 70 named viruses that are classified based on their antigenic, genomic and/or vector relationships into two groups: the Sandfly fever group and the Uukuniemi group (Nichol *et al.*, 2005). Viruses in the Sandfly fever group are transmitted by phlebotomine sandflies and mosquitoes; the Uukuniemi group viruses are tick-borne. Until recently, viruses in the Sandfly fever group were thought to be the only phleboviruses of public health or veterinary importance; however, three new Uukuniemi group viruses, severe fever with thrombocytopenia syndrome virus (Yu *et al.*, 2011), Heartland virus (McMullan *et al.*, 2012) and Bhanja virus (Matsuno *et al.*, 2013) were recently implicated in human disease. Phylogenetic analysis of these and other bunyaviruses has suggested the existence of a third distinct lineage (group)

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

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The GenBank/EMBL/DDBJ accession numbers for the sequences presented are listed in Table 1.

Two supplementary figures are available with the online version of this paper.

within the genus *Phlebovirus*, which is composed of Gouleako virus (Marklewitz *et al.*, 2011), and a second mosquito virus (Cumuto) from Trinidad (Auguste *et al.*, in press).

Seven named viruses are currently included within the Sandfly fever Naples species complex, based on their antigenic relationships: Sandfly fever Naples virus (SFNV), Toscana (TOSV), Massilia (MASV), Tehran virus (TEHV), Karimabad (KARV), Granada (GRV) and Punique (PUNV) (Nichol *et al.*, 2005). TOSV infection is a relatively common cause of meningitis and encephalitis in travellers to and residents of the Mediterranean region (Charrel *et al.*, 2005). SFNV is widely distributed in the eastern Mediterranean and Central Asia where it causes an acute febrile illness known as 'phlebotomus, sandfly or papatacci fever' that is characterized by severe headache, myalgia, conjunctival injection, malaise, nausea and vomiting, and a marked leucopenia of 3–5 days duration (Tesh, 1988). The other five viruses in the species complex (MASV, TEHV, KARV, GRV and PUNV) have only been isolated from phlebotomine sandflies and have not yet been associated with human disease, although there is serological evidence that some of them also infect people (Collao *et al.*, 2010; Tesh *et al.*, 1976, 1977).

Because of the public health importance of viruses in genus *Phlebovirus* and in an effort to develop a more precise taxonomic system for the phleboviruses, we have attempted to sequence all of the available named viruses in the genus to clarify their phylogenetic relationships. This is the fifth of a series of publications describing this work (Palacios *et al.*, 2011a, b, 2013a, b), and it covers members of the SFNV species complex.

Viruses used in this study were obtained from the World Reference Center for Emerging Viruses and Arboviruses at the University of Texas Medical Branch. Table 1 provides the names, strain numbers, sources, and dates and locality of isolation, and GenBank accession numbers. In addition to the seven Naples complex viruses noted before, three other tentative members, Saint Floris (SAFV), Gordil (GORV) and Gabek Forest (GFV) viruses, were also included.

Antisera for use in serological tests were prepared in adult mice as described previously (Palacios *et al.*, 2011b). The immunization schedule consisted of four intraperitoneal injections of mouse antigen mixed with Freund's adjuvant, given at weekly intervals. After the final immunization, mice were inoculated with sarcoma 180 cells, and the resulting immune ascitic fluids were collected.

Complement fixation (CF) tests were performed by the microtitre technique (Beaty *et al.*, 1989), using 2 units of guinea pig complement and overnight incubation of the antigens and antibodies at 4 °C. Haemagglutination-inhibition (HI) tests were also performed in microtitre plates, using 4 units of antigen and overnight incubation at 4 °C. Antigens used in the serological tests were prepared from infected newborn mouse brain by the sucrose acetone extraction method (Clarke & Casals, 1958) and were inactivated with 0.05 % β-propriolactone (Sigma) or by gamma irradiation. CF titres were recorded as the highest dilutions giving 3+ or 4+ fixation of complement on a scale of 0 to 4+. In HI tests using 4 units of antigen, antibody titres of 1 : 20 or greater were considered positive.

Results of CF and HI tests are shown in Table 2. By both tests SFNV, TEHV, TOSV and PUNV are closely related antigenically. In fact, except for PUNV, they are indistinguishable by CF test. Likewise, GORV and SAFV are closely related, although they can be differentiated from each other. Both GORV and SAFV also show considerable cross-reactivity with SFNV, TEHV, TOSV and PUNV, indicating that they belong to the Sandfly fever Naples species complex as well. In contrast, KARV and GFV are more distantly related antigenically to the other six viruses. KARV and GFV show some cross-reactivity with most of the Sandfly fever Naples complex viruses in HI tests, but not by CF test. KARV and GFV are more closely related to each other but are also distinct by these serological tests. Given this reactivity pattern, they appear to constitute a novel species complex within the genus *Phlebovirus*.

Genome sequencing was performed as previously described (Cox-Foster *et al.*, 2007; Palacios *et al.*, 2008, 2011b). For the termini of each segment, a primer with the 8 nt conserved sequence was used for a specific reverse transcription with an additional arbitrary nt on the 5′ end (5′-AAGCAGTGGTATCAACGCAGAGTAC**ACACAAAG**-3′) where the bold portion highlights the conserved region. This primer is designed to bind to the 3′ end of the genomic RNA and the 3′ of the mRNA. Sequences of the genomes were verified by classical dideoxy sequencing, using primers designed from the draft sequence to create products of 1000 bp with 500 bp overlaps.

Geneious 4.8.3 (Biomatters) was used for sequence assembly and analysis. Topology and targeting predictions were generated by employing SignalP, NetNGlyc, TMHMM (http://www.cbs.dtu.dk/services), the web-based version of TopPred2 (http://bioweb.pasteur.fr/seqanal/interfaces/toppred.html), and integrated predictions in Geneious (Bendtsen *et al.*, 2004; Claros & von Heijne, 1994; Kahsay *et al.*, 2005; Käll *et al.*, 2004; Krogh *et al.*, 2001).

The genome organization of all the viruses in the Sandfly fever Naples antigenic complex is consistent with other members of the genus *Phlebovirus*. In total, the genomes encode six proteins: encoding an RNA polymerase (L segment), two glycoproteins and a non-structural protein (G_N, G_C and NSm; M segment), and the nucleocapsid protein (N) and, in an ambisense orientation, a second non-structural protein (NSs; S segment). Several regions of the RNA-dependent RNA polymerase overlap conserved regions found in all available phlebovirus sequences, confirming an association with function (Palacios *et al.*, 2011a, b, 2013a, b). Signal sequences, transmembrane domains, cleavage sites for the cellular signal signallase protease

Table 1. Names, abbreviations, strain numbers, sources, dates and locality of isolation, and accession numbers of the viruses used in this study

Virus name	Abbreviation	Strain	Year of isolation	Source of isolate, location	Area of circulation (isolation, seroprevalence)	Accession numbers*
Saint Floris	SAFV	Dak ANBR 512d	1971	*Tatera* sp., Central African Republic	Central African Republic, Somalia, Sudan, Egypt	**JF920136 (L) JF920137 (M) JF920138 (S)**
Gordil virus	GORV	Dak ANBR 496d	1971	*Lemniscomys striatus,* Central African Republic	Central African Republic, Somalia, Sudan	**KF297900 (L) KF297901 (M) KF297902 (S)**
Sandfly Fever Naples	SFNV	R3	1985	Human serum, Cyprus	Cyprus, India, Italy, Turkey, Israel, Egypt, Bangladesh, Iran, Sudan, Ethiopia, Iraq, Pakistan, Turkmenistan,	**HM566183 (L) HM566184 (M) HM566182 (S)**
Sandfly Fever Naples	SFNV	Poona 701795	1971	Human serum, India	Azerbaijan, Saudi Arabia, Somalia	**HM566176 (L) HM566177 (M) HM566178 (S)**
Sandfly Fever Naples	SFNV	Sabin	1944	Human serum, Italy		**JF939843, HM566169 (L) JF939844, HM566171 (M) JF939845, HM566170 (S)**
Sandfly Fever Naples	SFNV	NAMRU 840055	1985	Human serum, Egypt		**HM566167 (L) HM566169 (M) HM566168 (S)**
Sandfly Fever Naples	SFNV	Yu 8/76	1976	*Phlebotomus perfiliewi*, Serbia	Serbia	**JF920139 (L) JF920140 (M) JF920141 (S)**
Tehran virus	TEHV	I-47	1959	*Phlebotomus papatasi*, Iran	Iran	**JF939846 (L) JF939847 (M) JF939848 (S)**
Granada	GRV	GRV25	2004	*Phlebotomus perniciosus,* man, Spain	Spain	GU135606 (L) GU135607 (M) GU135608 (S)
Massilia	MASV	W	2005	*Phlebotomus* spp., France	France	EU725771 (L) EU725772 (M) EU725773 (S)
Punique	PUNV	P1/B4	2008	*Phlebotomus perniciosus Phlebotomus longicuspis*	Tunis	**FJ848987 (L) FJ848988 (M) FJ848989 (S)**
Toscana	TOSV A	ISS.Phl.3	1984	*Phlebotomus perniciosus,* man, Italy	Italy, France, Spain, Greece, Portugal, Morocco, Tunisia, Bosnia-Herzegovina, Croatia, Kosovo, Turkey	X68414 (L) X89628 (M) X53794 (S)
Toscana	TOSV B	H/MTSSA				FJ153281 (L) Fj153283 (M) Fj153282 (S)
Karimabad	KARV	I-58	1959	*Phlebotomus* spp., Iran	Iran, Azerbaijan, Uzbekistan, Turkmenistan	**KF297912 (L) KF297913 (M) KF297914 (S)**
Karimabad	KARV	91019-P	1975	*Phlebotomus papatasi*, Iran	Kyngyzstan, Tajikstan, Russia	**KF297906 (L) KF297907 (M) KF297908 (S)**
Karimabad	KARV	91045-AG	1975	*Phlebotomus papatasi*, Iran		**KF297909 (L) KF297910 (M) KF297911 (S)**
Gabek Forest	GFV	Sud AN 754-61	1961	*Acomys cahirinus*, Sudan	Sudan, Senegal, Cent. African Rep., Nigeria, Benin	**KF297903 (L) KF297904 (M) KF297905 (S)**

*Bold font highlights sequences obtained in this work.

Table 2. Results of complement fixation (CF) and haemagglutination-inhibition (HI) tests

Antibody	Complement fixation test*							Haemagglutination-inhibition test†						
	Antigen							Antigen 4 units						
	SFN	TEH	TOS	PUN	GOR	SAF	KAR	GF	SFN	TEH	TOS	PUN	GOR	SAF	KAR	GF
SFN	**256/32**	256/32	256/32	64/32	0	0	0	0	**1:320**	1:160	1:80	1:80	1:40	1:40	1:40	1:40
TEH	64/128	**256/128**	128/32	32/32	0	0	0	0	1:2560	**1:2560**	1:320	1:320	1:320	1:320	1:160	1:40
TOS	64/128	256/128	**256/128**	32/32	0	0	0	0	1:640	1:640	**1:5120**	1:320	1:320	1:320	1:160	1:160
PUN	256/⩾8	256/⩾8	128/⩾8	**256/⩾8**	0	0	0	0	1:640	1:160	1:40	**1:2560**	1:40	1:80	1:40	1:20
GOR	0	16/8	32/8	16/⩾8	**512/⩾128**	32/⩾128	0	0	1:640	1:640	1:640	1:640	**1:5120**	1:640	1:40	1:40
SAF	0	8/8	16/8	16/⩾8	16/⩾128	**512/⩾128**	0	0	1:640	1:320	1:320	1:320	1:640	**1:2560**	0	0
KAR	0	0	0	0	0	0	**32/128**	0	1:40	1:20	1:40	1:40	0	1:20	**1:320**	1:160
GF	0	0	0	0	0	0	0	**256/128**	1:160	1:40	1:80	1:40	0	1:40	1:640	**1:1280**

SFN, Sandfly fever Naples (Sabin strain); TEH, Tehran; TOS, Toscana; PUN, Punique; KAR, Karimabad (I-58); GF, Gabek Forest; GOR, Gordil; SAF, Saint Floris.

*CF titres are expressed as the highest antibody dilution/highest antigen dilution, 0= <8/8. Bold numbers represent antibody reactivity with self-antigens.

†HI titres are expressed as the highest positive antibody dilution, 0= <1:20.

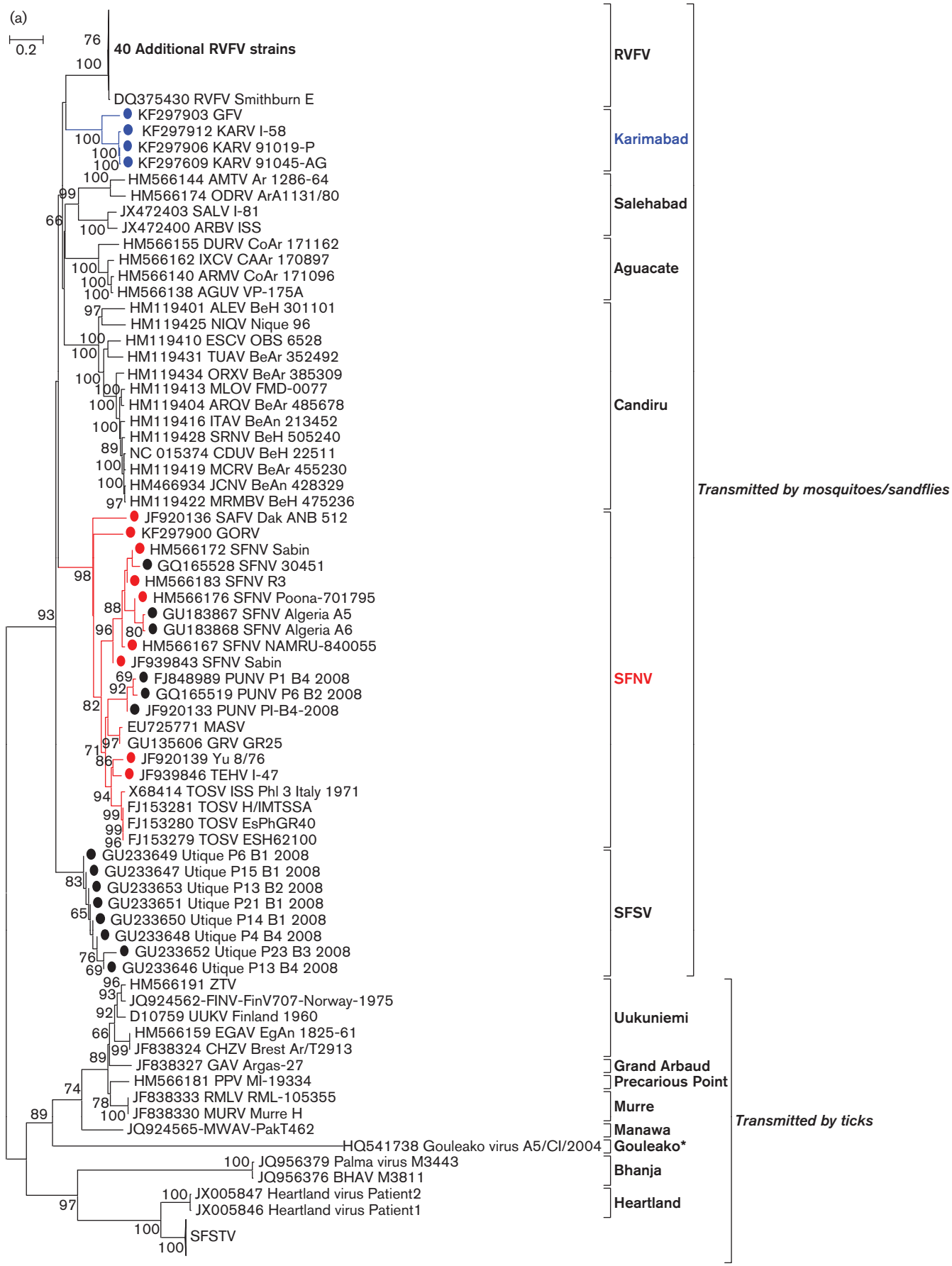
and Golgi retention signals for the G_N and G_C are conserved in the majority of the viruses in the SFNV virus group. In general, no major differences are observed between SFNV and other previously described phlebovirus (Palacios *et al.*, 2011a, b, 2013a, b). The only observation of note was the absence of a proper ORF for the NSs gene for SFNV strain YU8-76 and TEHV strain I-47. An early stop codon (aa position 215, strain Yu8-76) and a gene truncation (region deleted: 101–270 aa, strain I-47) are observed in the NSs genes of these two isolates.

For phylogenetic analysis, a set of phlebovirus sequences (131 for the L segment, 169 for the M segment, 191 for the N gene, and 146 for the NS gene) comprising all nt (partial or complete) sequences from GenBank available on May 1, 2013 were aligned, along with our sequences, using the CLUSTAL algorithm (as implemented in the MEGA package Version 5) at the aa level with additional manual editing to ensure the highest possible quality of alignment. Neighbour-joining (NJ) analysis at the aa level was performed due to the observed high variability of the underlying nt sequences. Given the saturation observed in all the alignments, the phylogenetic trees obtained by analysis of all members of the genus were used to define the species complexes, while additional phylogenetic analysis restricted to the SFNV sequences was used to resolve the fine topology of the species complex.

The statistical significance of tree topology was evaluated by bootstrap resampling of the sequences 1000 times. Phylogenetic analyses were performed by using MEGA software (Tamura *et al.*, 2011).

Phylogenetic analyses of the L, M and S gene segment sequences of the 12 new viruses (SAFV, GORV, SFNV strain R3, Poona 701795, Sabin, NAMRU 840055, Yu 8/76, TEHV strain I-47, KARV strain I-58, 91019-P and 91045-AG, and GFV) are consistent with earlier reports, confirming that viruses belonging to the same group cluster together (Charrel *et al.*, 2009; Collao *et al.*, 2010). As anticipated, based on their cross-reactivity in CF tests (Bishop *et al.*, 1980), members of the SFNV species complex generally cluster together with the exception of KARV, which forms its own clade along with the previously uncharacterized GFV (Figs 1 and S1, available in JGV Online). Based on L-, M-, and S-segment sequences, the viruses that form a monophyletic clade with SFNV cluster into five groups: Toscana, Sandfly fever Naples, Yu 8/76/Tehran, Punique/Massilia/Granada and Gordil/Saint Floris. It appears that KARV and GFV show only distant evolutionary relationships with the other members of the SFNV serogroup. Both viruses formed a single clade that has similar levels of diversity compared to other phlebovirus species (data not shown).

Systematic screening for the presence of recombination patterns was pursued by using the nt alignments and the Recombination Detection Program (RDP, (Martin & Rybicki, 2000) and Bootscan (Salminen *et al.*, 1995), MaxChi (Smith, 1992), Chimaera (Posada & Crandall, 2001), LARD (Holmes, 1998) and PHYLIP Plot (Felsenstein, 1989).


(a)
0.2
40 Additional RVFV strains
DQ375430 RVFV Smithburn E
KF297903 GFV
KF297912 KARV I-58
KF297906 KARV 91019-P
KF297609 KARV 91045-AG
HM566144 AMTV Ar 1286-64
HM566174 ODRV ArA1131/80
JX472403 SALV I-81
JX472400 ARBV ISS
HM566155 DURV CoAr 171162
HM566162 IXCV CAAr 170897
HM566140 ARMV CoAr 171096
HM566138 AGUV VP-175A
HM119401 ALEV BeH 301101
HM119425 NIQV Nique 96
HM119410 ESCV OBS 6528
HM119431 TUAV BeAr 352492
HM119434 ORXV BeAr 385309
HM119413 MLOV FMD-0077
HM119404 ARQV BeAr 485678
HM119416 ITAV BeAn 213452
HM119428 SRNV BeH 505240
NC 015374 CDUV BeH 22511
HM119419 MCRV BeAr 455230
HM466934 JCNV BeAn 428329
HM119422 MRMBV BeH 475236
JF920136 SAFV Dak ANB 512
KF297900 GORV
HM566172 SFNV Sabin
GQ165528 SFNV 30451
HM566183 SFNV R3
HM566176 SFNV Poona-701795
GU183867 SFNV Algeria A5
GU183868 SFNV Algeria A6
HM566167 SFNV NAMRU-840055
JF939843 SFNV Sabin
FJ848989 PUNV P1 B4 2008
GQ165519 PUNV P6 B2 2008
JF920133 PUNV PI-B4-2008
EU725771 MASV
GU135606 GRV GR25
JF920139 Yu 8/76
JF939846 TEHV I-47
X68414 TOSV ISS Phl 3 Italy 1971
FJ153281 TOSV H/IMTSSA
FJ153280 TOSV EsPhGR40
FJ153279 TOSV ESH62100
GU233649 Utique P6 B1 2008
GU233647 Utique P15 B1 2008
GU233653 Utique P13 B2 2008
GU233651 Utique P21 B1 2008
GU233650 Utique P14 B1 2008
GU233648 Utique P4 B4 2008
GU233652 Utique P23 B3 2008
GU233646 Utique P13 B4 2008
HM566191 ZTV
JQ924562-FINV-FinV707-Norway-1975
D10759 UUKV Finland 1960
HM566159 EGAV EgAn 1825-61
JF838324 CHZV Brest Ar/T2913
JF838327 GAV Argas-27
HM566181 PPV MI-19334
JF838333 RMLV RML-105355
JF838330 MURV Murre H
JQ924565-MWAV-PakT462
HQ541738 Gouleako virus A5/CI/2004
JQ956379 Palma virus M3443
JQ956376 BHAV M3811
JX005847 Heartland virus Patient2
JX005846 Heartland virus Patient1
SFSTV
RVFV
Karimabad
Salehabad
Aguacate
Candiru
SFNV
SFSV
Uukuniemi
Grand Arbaud
Precarious Point
Murre
Manawa
Gouleako*
Bhanja
Heartland
Transmitted by mosquitoes/sandflies
Transmitted by ticks

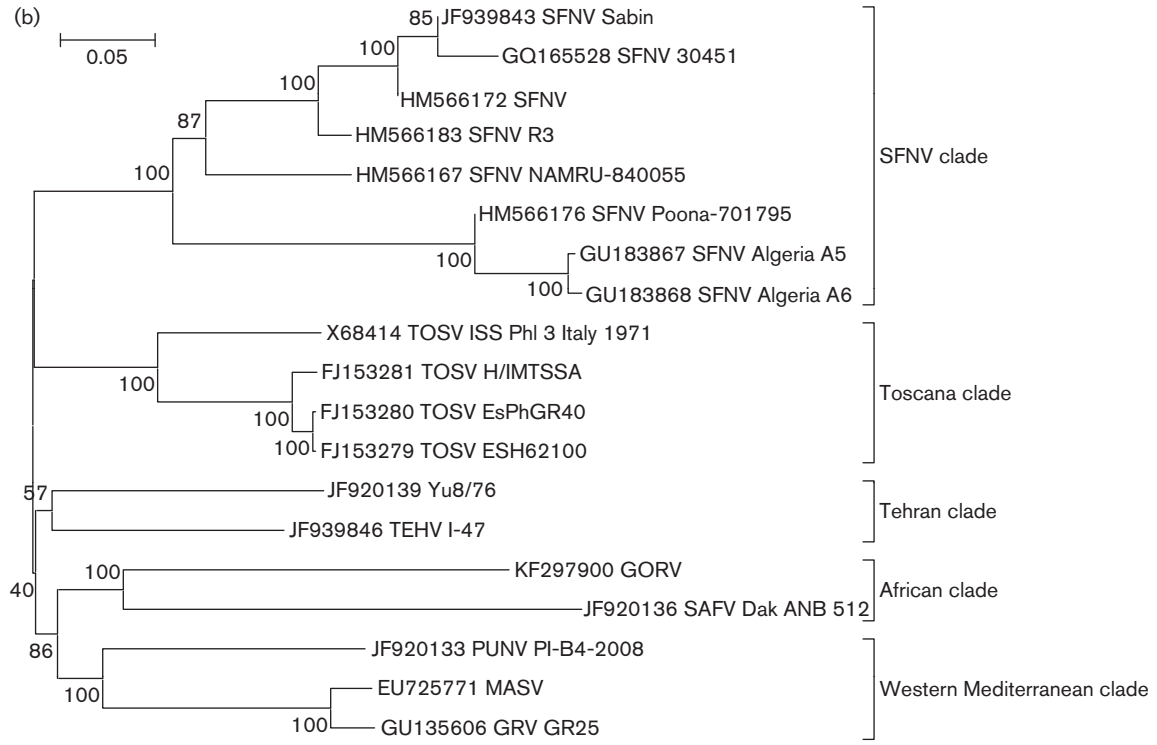


Fig. 1. (a) Phylogenetic analysis of the available sequences of phlebovirus L ORF. Sequences marked with black dots corresponded to partial sequences. Sequences marked with red dots (Sandfly fever Naples species complex) and blue dots (Karimabad species complex) corresponded to sequences obtained during this work. Only partial (when only available for the species) or complete ORF sequences were included in the analysis. Non-coding regions were excluded. *Gouleako virus was actually recovered from mosquitoes. (b) Phylogenetic analysis of all members of the Sandfly fever Naples species complex L segments. Significant bootstrapping (>65) values are indicated at each branch point. Scale bars show the number of amino acid substitutions per site.

With the known exception of GRV (Collao *et al.*, 2010), no evidence of SFNV reassortment was found in topological analysis of phylogenetic trees (Figs 2 and S2) or by RDP, Bootscan, MaxChi, LARD and PHYLIP Plot analysis (data not shown).

Phylogenetic analysis of the complete genomes of the isolates of the Sandfly fever Naples species complex allowed us to identify geographical and genetic correlations. The African viruses SAFV and GORV, previously reported to be related based on serological criteria (Tesh *et al.*, 1976, 1982), form one clade. GRV, MASV and PUNV, all recently discovered viruses detected in the western Mediterranean region, form a second phylogenetic cluster, suggesting a common ancestor. TOSV, a human pathogen detected throughout the Mediterranean region forms a third clade. The widely distributed SFNV isolates from Cyprus, Italy, Egypt and India form a fourth clade. TEHV and the Serbian isolate Yu 8/76 form a fifth clade. In this new classification scheme, strains R3, Poona 701795, Sabin and NAMRU 840055 would be representatives of the SFNV clade, while I-47 (TEHV) and Yu 8/76 would be representatives of a new TEHV clade. Interestingly, this last clade apparently does not require expression of the NSs ORF, since its replication is not impaired by the presence of either an early stop codon or a large truncation. NSs proteins have been reported to be an important determinant in the pathogenesis of phleboviruses (Billecocq *et al.*, 2004; Bouloy *et al.*, 2001; Ikegami *et al.*, 2006; Sall *et al.*, 1997; Vialat *et al.*, 2000). The main supporting argument for this statement is the finding and description of a naturally attenuated RVFV strain (clone 13) that has a large in-frame deletion in the NSs coding region of the S segment (Muller *et al.*, 1995). The truncated NSs protein of clone 13 is expressed and remains in the cytoplasm, where it is degraded rapidly by the proteasome.

KARV and GFV are distinct and form a new species complex within the Sandfly fever group. KARV was originally isolated in Iran in 1959 (Berge, 1975); subsequent KARV isolations and serological evidence of human infection have been reported from Iran, Azerbeijan, Uzbekistan, Turkmenistan, Kyrgyzstan, Tajikistan and Russia (Gaidamovich *et al.*, 1991; Tesh *et al.*, 1976, 1977). GFV was initially isolated in Sudan in 1961 but many subsequent isolates have been obtained in Nigeria, Benin, Senegal and Central African Republic (Kemp *et al.*, 1974). Serological evidence of human infection with GFV has been reported in Sudan, Egypt and Nigeria (Tesh *et al.*, 1976). Since KARV was the first of the two viruses to

be isolated, Karimabad should be the species designation of this new complex.

Segment reassortment in bunyaviruses has been reported with increasing frequency, especially in the genus *Orthobunyavirus* (Bowen *et al.*, 2001; Briese *et al.*, 2006, 2007; Burt *et al.*, 2009; Collao *et al.*, 2010; Iroegbu & Pringle, 1981; Kondiah *et al.*, 2010; Nunes *et al.*, 2005; Saeed *et al.*, 2001; Yanase *et al.*, 2006, 2010). Previously, we reported that the frequency of reassortment in the Candiru species complex of the genus *Phlebovirus* (five of thirteen named viruses) was unprecedented (Palacios *et al.*, 2011a). In contrast, our analysis of members of the Uukuniemi group did not indicate any reassortment events (Palacios *et al.*, 2013b). With the exception of the aforementioned reassortment of GRV, no additional reassortment was detected among members of the SFNV species group.

The antigenic relatedness between KARV and some members of the SFNV species complex illustrates one of the problems of bunyavirus classification, using serological data from a single type of test (i.e. HI or CF). There is sometimes a poor correlation between results of CF and HI tests. In the case of the phleboviruses, the HI tends to be broadly reactive, whereas the CF test is more species-specific. The results of CF tests generally correlate more closely with the genetic data. The relationship of KARV with SFNV is an example. Other examples of this discordance within the genus *Phlebovirus* are the relationships between the Uukuniemi, Bhanja and Severe fever with thrombocytopenia species groups (Matsuno *et al.*, 2013; Palacios *et al.*, 2013b), and the relationships observed within the Salehabad species group (Palacios *et al.*, 2013a). For this reason, we previously suggested that a classification system for the family *Bunyaviridae* should be based on genetic as well as serological (CF) data (Palacios *et al.*, 2013b).

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