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Characterization of new small ruminant lentivirus subtype B3 suggests animal trade within the Mediterranean Basin

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Abstract
Small ruminant lentiviruses (SRLVs) represent a group of viruses infecting sheep and goats worldwide. Despite the high heterogeneity of genotype A strains, which cluster into as many as ten subtypes, genotype B was believed to be less complex and has, so far, been subdivided into only two subtypes. Here, we describe two novel full-length proviral sequences isolated from Sarda sheep in two Italian regions. Genome sequence as well as the main linear epitopes clearly placed this cluster into genotype B. However, owing to long-standing segregation of this sheep breed, the genetic distances that are clearly .15 % with respect to B1 and B2 subtypes suggest the designation of a novel subtype, B3. Moreover the close relationship with a gag sequence obtained from a Turkish sheep adds new evidence to historical data that suggest an anthropochorous dissemination of hosts (small ruminants) and their pathogens (SRLV) during the colonization of the Mediterranean from the Middle East.

Main text
The small ruminant lentiviruses (SRLVs) represent a very heterogeneous group of viruses infecting goat and sheep. Based on a limited number of complete sequences, they were initially described as two distinct genetic groups evolving independently in sheep or goats, with the ovine strains being closely related to each other and distinct from the caprine ones.
Over the past two decades, the description and phylogen- etic analysis of many partial or complete sequences of caprine and ovine field isolates from various geographical regions have clearly highlighted the existence of a genetic continuum, with viruses that did not simply cluster according to the animal species they were isolated from. To date, SRLVs have been classified into five genotypes. At east three genotypes are well represented in Italy: genotype A, which includes the visna maedi virus (VMV) cluster, genotype B, which includes caprine arthritis encephalitis virus (CAEV) and genotype E, thus far described only in Italy as two distinct subtypes and which are strictly associated with the goat population and widely distributed in Sardinia (Grego et al., 2007; Reina et al., 2011).
With the exception of genotypes C, D and E, which seem to be geographically restricted to limited areas, genotypes A and B have been described worldwide with well-known associated diseases.

Highly heterogeneous VMV-like strains cluster into as many as ten subtypes while genotype B seems to be less complex and has so far been subdivided into only two subtypes, B1 and B2 (Leroux et al., 2010; Pisoni et al., 2010). The former (prototype strain CAEV Cork, Saltarelli et al., 1990) which is associated with arthritis, mastitis, encephalitis and pneumonia, was originally detected in some goat breeds such as the French and Swiss Alpine and the Saanen. The wide distribution of B1 has been associated with extensive animal trading owing to the improved milk production of these breeds. Subtype B2 (prototype strain 492, Glaria et al., 2009) has been detected in several sheep breeds in Europe and is associated with pneumonia, arthritis and mastitis (Glaria et al., 2009; Grego et al., 2002; Valas et al., 1997).

Owing to the antigenic heterogeneity of some linear epitopes within gag products, animals infected with genotypes A, B or E can be conveniently serotyped based on differential reactivity against the corresponding antigen subunit, representing a simple and cost-effective alternative to genetic analysis. In this context, serological surveys carried out in the past decade revealed that several Italian sheep flocks were infected with CAEV-like and E strains (Grego et al., 2002, 2005; Reina et al., 2011).

In a previous study, a flock of Sarda sheep located near Volterra (Tuscany region, Italy), which was showing clinical signs of mastitis and pneumonia, was found to be serologically reactive against genotype B epitopes. However, the virus isolated from a seropositive sheep, although characterized as CAEV-like by phylogenetic analysis of the pol region, could not be assigned to the previously described clusters, suggesting that the genetic heterogeneity of genotype B could be more complex than previously thought (Grego et al., 2005). More recently, a serological survey carried out in Sardinia has revealed that Sarda sheep flocks were mainly infected with genotype B strains (Reina et al., 2010), and partial pol sequences revealed substantial similarity with the Volterra isolate (S. Dei Giudici, personal observation). Since the Sarda sheep represent the major Italian sheep breed, accounting for more than 4 million head, approximately half of which are reared in Sardinia and the rest in central Italy, the full genetic characterization of Sarda sheep-associated lenti- viruses may be important from a biological and diagnostic point of view.

In this study, we present the full genomic characterization of two strains obtained from distinct regions (Sardinia and Tuscany, Italy), providing evidence for the circulation of a new subtype, B3, infecting sheep and goat. Moreover a substantial similarity with a Turkish SRLV gag sequence was found, thus suggesting an alternative route of SRLV dissemination during the Mediterranean colonization.

The ovine Sardinia 22921-Co4 isolate, named Fonni, was obtained from a seropositive sheep by co-cultivation of blood monocyte-derived macrophages (BMDM) with ovine choroid plexus cells. The isolate Volterra, obtained from lung tissue explants from a seropositive sheep showing signs of mastitis and pneumonia, was propagated on ovine fetal lung fibroblasts (OFL). Supernatants from cultures showing cytopathic effect were aliquoted and stored at 280 uC. Proviral DNA of both isolates was extracted from infected cultures and amplified with universal and sequence-derived primer sets. All the primer sequences used in this study are available upon request. Overlapping amplicons spanning the whole proviral genome were cloned into pCR2.1-TOPO TA vector (Invitrogen) and were sequenced in both strands or
directly sequenced (BMR genomics). Additional partial gag and pol sequences were obtained from sheep and goats from Sardinia flocks using the primer set and previously described conditions (Eltahir et al., 2006; Grego et al., 2007). All of the new sequences clustered together with Fonni and Volterra B3 prototypes, showing an overall divergence of 16.01 % (gag partial sequence) and 16.91

% (pol partial sequence). A 1.3 kb gag complete gene sequence was obtained from a Turkish sheep in a previous study (strain TR-DM, unpublished data).
The overall nucleotide and amino acid divergences between Fonni and Volterra strains, expressed as the mean proportion of differences among sequences (Nei, 1987), were 17.89 and 14.77 %, respectively. Nucleotide and amino acid similarity to reference genomes were evaluated and are reported in Table 1. All phylogenetic analyses were performed by selecting the best molecular evolutionary model using the ModelTest software (Posada & Buckley, 2004), and creating phylogenetic trees using Bayesian approaches implemented in the MrBayes software (Ronquist & Huelsenbeck, 2003). In particular, the phylogenetic tree obtained by using all concatenated genes was built and included the three different molecular evolutionary models in the partitioned dataset of sequences.
The viral genome of the Fonni isolate consists of 9148 nt and includes gag (1320 nt), pol (3201 nt), vif (687 nt), tat (264 nt) and env (2814 nt) coding regions. Rev protein is encoded by two exons (117 and 297 nt), which are both included in the env gene sequence. The Volterra isolate viral genome is 9152 bp and has a very similar organiza- tion, including gag (1320 nt), pol (3339 nt), vif (690 nt), tat (264 nt) and env (2808 nt) coding regions. Rev protein is encoded by two exons (120 and 285 nt), which are both included in the env gene sequence. Since the viral sequences were obtained by overlapping PCR fragments, they may not represent the sequence of a single provirus. However, their genetic organization reflects that of the classical replication-competent SRLVs prototypes.
In order to gain some insight into the biological features of these new viral strains, the U3 region was analysed. The Fonni and Volterra strains share very similar organization of the promoter region, which contains the highly con- served AML (acute myeloid leukaemia) motif (Murphy et al., 2010) and the AP4 tandem repeat, which has been found to be necessary and sufficient for efficient transcrip- tional activation (Juganaru et al., 2010; Mermod et al., 1988). The tumour necrosis factor (TNF)-activated tran- scription site (Murphy et al., 2007) and the AP1 sequence (Gabuzda et al., 1989) were slightly heterogeneous compared with CAEV strains (data not shown). A putative CAAAT motif is present in the Volterra promoter and is identical to the one described in visna virus (Hess et al., 1985), whereas gamma-activated sites were absent in both isolates. Interestingly, the LTR of pulmonary and neuro- logical VMV strains carries one and two copies, respec- tively, of the CAAAT motif, suggesting a role in the onset of respiratory and neurological disease (Oskarsson et al., 2007). Therefore, the pathogenic potential of the strains characterized in the present study needs to be carefully evaluated.
Phylogenetic analysis was performed by alignment of the newly obtained sequences with reference genomes. The overall nucleotide diversity for each gene (Table 1) supported the identification of the new isolates as members of genotype B. However, owing to the substantial genetic distances (clearly .15 %) between the Fonni and Volterra genomes with respect to the B1 and B2 subtypes, a third B subtype named B3 was proposed. The establishment of this new subtype fulfils the criteria recommended in the human immunodeficiency virus field, whereby at least two epidemiologically unlinked isolates should be sequenced in their entirety (Robertson et al., 1999). Moreover, the circulation of this new subtype is confirmed by partial gag and pol sequences from several caprine and ovine samples obtained in this study and collected in the Sardinia region. All new sequences were deposited in GenBank. The assignment to genotype B was also strongly supported by the similarity of the immunodominant regions; in fact, Fonni and Volterra presented CAEV-like epitopes in gagMA, gagCA and envTM sequences (Table 2). While the sequence of env

hypervariable region 1 (HV1) maintained the typical pattern GNXT, differences were also found in hypervariable region 2 (HV2, Table 2). High heterogeneity is also clear in the SU5 amino acid sequence, supporting the high specificity of this immunodominant region (Carrozza et al., 2009). The phylogenetic tree based on concatenated gag, pol and env genes (Fig. 1a) clusters the new isolates within the genotype B clade, confirming the existence of the novel subtype. The same tree topology was obtained using only complete gag sequences. Of note is the close relationship between the new isolates and the Turkish ovine isolate TR-DM (Fig. 1b). There is no clear evidence of animal trade between Turkey and Sardinia in
the recent past. However, the close similarity of the Turkish sequence to the two Italian sequences seems to indicate recent segregation of these viruses. Additional sequence data will be necessary to support the phylogenetic clade structure and to clarify the relationships among these viral strains. There is a substantial amount of data suggesting that this might have occurred during the Neolithic age following colonization of the Mediterranean. Moreover, the recent finding that bulk milk samples from Turkish sheep and goats were reactive against antigen derived from genotype E (D. Muz, unpublished personal observation), which to date has been found strictly associated with goat species in Sardinia (Reina et al., 2010), further supports this ancient epidemiological linkage. This point is particu- larly interesting because a fascinating hypothesis is emerging: since the goat is believed to be the first domesticated and anthropochorous mammal of the Mediterranean Islands (Masseti, 2009; Naderi et al., 2007) it could be argued that segregation of the goats for centuries in Sardinia may have led to increased fitness of the animals infected with genotype E compared with those infected with other viral genotypes. This aspect is possibly because of the reduced virulence of genotype E, characterized by dUTPase and VPR-like deletion, its limited or impaired cross-species transmission and its ability to trigger an immune response against SRLVs of other genotypes (Reina et al., 2011), thus avoiding clinical disease. Although further studies are needed to assess the following hypothesis, it is conceivable that type B virus (specifically subtype B3 in Sardinia) was introduced to Sardinia through infected domesticated sheep (which originated later than domesticated goats) (Masseti, 2009) while maintaining its capacity to infect sheep and goats, as suggested by the characterization of several B3 gag and pol sequences.
Specifically, to determine the ability of the viral isolate Fonni to infect caprine cells and lead to a productive infection, an entry assay was performed using a viral pseudotype expressing the envelope protein of Fonni. The env gene was amplified with primers carrying a HindIII restriction site and a functional Kozac sequence (forward primer), and a NotI restriction site (reverse primer). The amplicon was digested, gel purified and cloned between the HindIII and NotI restriction sites of a pCMVlcn expression vector (Ho ̈tzel & Cheevers, 2001) to yield the pCMV/Fonni plasmid. pCAEV-AP is a replication-deficient CAEV proviral clone that expresses the thermostable human placenta alkaline phosphatase (HPAP) reporter gene, which can be pseudotyped with the envelope glycoprotein of any small- ruminant lentivirus, allowing direct determination and comparison of species tropism for different strains (Ho ̈tzel & Cheevers, 2003). CAEV-AP pseudotyped with the envelope of the Fonni strain was produced by co-transfecting 16106 HEK-293T cells in six-well plates as previously described (Juganaru et al., 2011). Pseudotyped virus was collected 40 h post-transfection, clarified by centrifugation and frozen in aliquots at 280 uC. Subconfluent goat synovial membrane, OFL and BMDM cell cultures in 24-well plates were inoculated with 200 ml of serial 1 : 10 dilutions of CAEV-AP/Fonni. Following 2 h incubation at room temperature with occasional agitation, 1 ml of DMEM (Sigma)/10 % FBS (Invitrogen) was added to the cells and incubation proceeded for 72 h at 37 uC. Foci of infected cells were identified by HPAP staining as

described (Ho¨tzel & Cheevers, 2003). Titres were expressed as focus-forming units (f.f.u.) per millilitre. The entry assay clearly indicated that the envelope protein derived from the Fonni strain allowed efficient entry in both sheep and goat target cells, using a still-unknown cellular receptor(s) and producing a mean of 6.446103 f.f.u. ml21 in BMDM and from 16104 (caprine) to 2.46104 (ovine) f.f.u. ml21 in fibroblast-like cells. This is in agreement with the typical features of genotype B subtypes, which are able to pass the cross-species barrier (Pisoni et al., 2005; Germain & Valas, 2006).

In conclusion, we report the full genome proviral sequences of two SRLV isolates associated with the Sarda sheep breed in Italy and propose the designation of the new subtype B3 within the CAEV-like cluster. While the pathogenic potential of this subtype needs to be deter- mined, the infection of both sheep and goat with subtype B3 can be diagnosed using currently available serological tests, owing to the antigenic similarity with existing subtypes. The evidence of a Turkish SRLV gag sequence clustering with subtype B3 supports an ancient epidemio- logical linkage within the Mediterranean basin. From this perspective it would be very interesting to increase our knowledge of the genetic background and evolution of SRLVs in the Fertile Crescent area where small ruminant domestication occurred.

The increasing number of SRLV sequences from the geographical areas still unexplored in SRLV phylogenetic studies will require reassessment of the classification of these viruses.

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References

Carrozza, M. L., Mazzei, M., Lacerenza, D., Del Chiaro, L., Giammarioli, M., Marini, C., Rutili, D., Rosati, S. & Tolari, F. (2009). Seroconversion against SU5 derived synthetic peptides in sheep experimentally infected with different SRLV genotypes. Vet Microbiol 137, 369–374.

Eltahir, Y. M., Dovas, C. I., Papanastassopoulou, M., Koumbati, M., Giadinis, N., Verghese-Nikolakaki, S. & Koptopoulos, G. (2006). Development of a semi-nested PCR using degenerate primers for the generic detection of small ruminant lentivirus proviral DNA. J Virol Methods 135, 240–246.

Gabuzda, D. H., Hess, J. L., Small, J. A. & Clements, J. E. (1989). Regulation of the visna virus long terminal repeat in macrophages involves cellular factors that bind sequences containing AP-1 sites. Mol Cell Biol 9, 2728–2733.

Germain, K. & Valas, S. (2006). Distribution and heterogeneity of small ruminant lentivirus envelope subtypes in naturally infected French sheep. Virus Res 120, 156–162.

Glaria, I., Reina, R., Crespo, H., de Andre´ s, X., Ram´ırez, H., Biescas, E., Pe´ rez, M. M., Badiola, J., Luja´ n, L. & Amorena, B. (2009). Phylogenetic analysis of SRLV sequences from an arthritic sheep outbreak demonstrates the introduction of CAEV-like viruses among Spanish sheep. Vet Microbiol 138, 156–162.

Grego, E., Profiti, M., Giammarioli, M., Giannino, L., Rutili, D., Woodall, C. & Rosati, S. (2002). Genetic heterogeneity of small ruminant lentiviruses involves immunodominant epitope of capsid antigen and affects sensitivity of single-strain-based immunoassay. Clin Diagn Lab Immunol 9, 828–832.

Grego, E., Bertolotti, L., Carrozza, M. L., Profiti, M., Mazzei, M., Tolari, F. & Rosati, S. (2005). Genetic and antigenic characterization of the matrix protein of two genetically distinct ovine lentiviruses. Vet Microbiol 106, 179–185.

Grego, E., Bertolotti, L., Quasso, A., Profiti, M., Lacerenza, D., Muz, D. & Rosati, S. (2007). Genetic characterization of small ruminant lentivirus in Italian mixed flocks: evidence for a novel genotype circulating in a local goat population. J Gen Virol 88, 3423–3427.

Hess, J. L., Clements, J. E. & Narayan, O. (1985). cis- and trans-acting transcriptional regulation of visna virus. Science 229, 482–485.

Hotzel, I. & Cheevers, W. P. (2001). Host range of small-ruminant lentivirus cytopathic variants determined with a selectable caprine arthritis- encephalitis virus pseudotype system. J Virol 75, 7384–7391.

Hotzel, I. & Cheevers, W. P. (2003). Caprine arthritis-encephalitis virus envelope surface glycoprotein regions interacting with the transmembrane glycoprotein: structural and functional parallels with human immunodeficiency virus type 1 gp120. J Virol 77, 11578– 11587.

Juganaru, M., Reina, R., Grego, E., Profiti, M. & Rosati, S. (2010). LTR promoter activity of SRLV genotype E, strain Roccaverano. Vet Res Commun 34 (Suppl. 1), S47–S51.

Juganaru, M., Reina, R., Bertolotti, L., Stella, M. C., Profiti, M., Armentano, M., Bollo, E., Amorena, B. & Rosati, S. (2011). In vitro properties of small ruminant lentivirus genotype E. Virology 410, 88–95.

Leroux, C., Cruz, J. C. & Mornex, J. F. (2010). SRLVs: a genetic continuum of lentiviral species in sheep and goats with cumulative evidence of cross species transmission. Curr HIV Res 8, 94–100.

Masseti, M. (2009). The wild goats Capra aegagrus Erxleben, 1777 of the Mediterranean Sea and the Eastern Atlantic Ocean islands. Mammal Rev 39, 141–157.

Mermod, N., Williams, T. J. & Tjian, R. (1988). Enhancer binding factors AP-4 and AP-1 act in concert to activate SV40 late transcription in vitro. Nature 332, 557–561.

Murphy, B., Jasmer, D. P., White, S. N. & Knowles, D. (2007). Localization of a TNF-activated transcription site and interactions with the gamma activated site within the CAEV U3 70 base pair repeat. Virology 364, 196–207.

Murphy, B., McElliott, V., Vapniarsky, N., Oliver, A. & Rowe, J. (2010). Tissue tropism and promoter sequence variation in caprine arthritis encephalitis virus infected goats. Virus Res 151, 177–184.

Naderi, S., Rezaei, H. R., Taberlet, P., Zundel, S., Rafat, S. A., Naghash, H. R., el-Barody, M. A., Ertugrul, O., Pompanon, F. & Econogene Consortium (2007). Large-scale mitochondrial DNA analysis of the domestic goat reveals six haplogroups with high diversity. PLoS ONE 2, e1012.

Nei, M. (1987). Molecular Evolutionary Genetics. New York: Columbia University Press.

Oskarsson, T., Hreggvidsdo´ ttir, H. S., Agnarsdo´ ttir, G. Matth´ıasdo´ ttir, S., Ogmundsdo´ ttir, M. H., Jo´ nsson, S., Georgsson, G., Ingvarsson, S., Andre´ sson, O. S. & Andre´ sdo´ ttir, V. (2007). Duplicated sequence motif in the long terminal repeat of maedi-visna virus extends cell tropism and is associated with neurovirulence. J Virol 81, 4052–4057.

Pisoni, G., Quasso, A. & Moroni, P. (2005). Phylogenetic analysis of small-ruminant lentivirus subtype B1 in mixed flocks: evidence for natural transmission from goats to sheep. Virology 339, 147–152.

Pisoni, G., Bertoni, G., Manarolla, G., Vogt, H. R., Scaccabarozzi, L., Locatelli, C. & Moroni, P. (2010). Genetic analysis of small ruminant lentiviruses following lactogenic transmission. Virology 407, 91–99.

Posada, D. & Buckley, T. R. (2004). Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and bayesian approaches over likelihood ratio tests. Syst Biol 53, 793–808.

Reina, R., Bertolotti, L., Dei Giudici, S., Puggioni, G., Ponti, N., Profiti, M., Patta, C. & Rosati, S. (2010). Small ruminant lentivirus genotype E is widespread in Sarda goat. Vet Microbiol 144, 24–31.

Reina, R., Juganaru, M. M., Profiti, M., Cascio, P., Cerruti, F., Bertolotti, L., De Meneghi, D., Amorena, B. & Rosati, S. (2011). Immunological parameters in goats experimentally infected with SRLV genotype E, strain Roccaverano. Vet Immunol Immunopathol 139, 237–244.

Robertson, D. L., Anderson, J., Bradac, J. A., Carr, J. K., Foley, B., Funkhouser, R. K., Gao, F., Hahn, B. H., Kalish, M. & other authors (1999). HIV-1 nomenclature proposal: a reference guide to HIV-1 classification. The Human Retroviruses and AIDS 1999 Compendium Los Alamos National Laboratory, Los Alamos, 492–505.

Ronquist, F. & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572– 1574.

Saltarelli, M., Querat, G., Konings, D. A., Vigne, R. & Clements, J. E. (1990). Nucleotide sequence and transcriptional analysis of molecular clones of CAEV which generate infectious virus. Virology 179, 347– 364.

Valas, S., Benoit, C., Guionaud, C., Perrin, G. & Mamoun, R. Z. (1997). North American and French caprine arthritis-encephalitis viruses emerge from ovine maedi-visna viruses. Virology 237, 307– 318.

Table 1. Genetic divergence

The figures are uncorrected *P* distances from reference strains reported as percentages. NA, Not applicable because no complete genomes are available.

Strain	GenBank accession no.	Genotype or Subtype	*gag*			*pol*		*env*	
			Fonni	Volterra	TR-DM	Fonni	Volterra	Fonni	Volterra
Volterra (Fonni)	JF502416 (JF502417)	B3	12.93	0.00	13.01 (13.14)	18.04	0.00	20.14	0.00
CAEV Cork	M33677	B1	19.52	21.25	20.31	24.04	24.27	29.60	29.34
It-Pi1	AY265456	B2	19.65	27.92	21.90	NA	NA	NA	NA
Ov496	FJ195346	B2	19.89	19.70	18.94	22.53	22.82	27.97	28.33
1GA	AF322109	C	25.76	26.06	25.27	23.93	25.08	38.37	38.25
K1514	M60610	A	27.84	27.33	27.29	22.90	24.71	32.86	32.93
kv1772	L06906	A	27.68	27.17	27.28	22.83	24.68	33.11	33.30
SAOMVV	M31646	A	26.57	26.61	26.75	22.93	22.86	31.61	32.44
It-561	AY265455	A	20.70	27.99	28.64	NA	NA	NA	NA
Roccaverano	EU293537	E1	32.45	32.51	33.57	28.86	27.89	38.80	38.44
Seui	GQ381130	E2	31.77	32.61	33.02	28.23	28.58	36.38	36.60

Table 2. Immunodominant regions

Epitopes and immunodominant regions are compared among new and references strains. *gag*MA, Matrix protein epitope within *gag*; *gag*CA, capsid protein epitope within *gag*; *env*TM, transmembrane domain within *env*; *env*HV1 and *env*HV2, hypervariable (HV) regions within *env*; *env*SU5, SU5 epitope within *env*. HV regions HV1 and HV2 are in boldface type. NA, Not applicable because no complete genomes are available.

Strain	Accession no.	Genotype	*gag*MA	*gag*CA	*env*TM	*env*HV1	*env*HV2	*env*SU5
Fonni	JF502416	B3	KELTPEESNKKDYASL	KLNEEAERWRRNNPPPAA	QELDCWHYHQYCVTS	V**GNNT**VTGNCSVHN	KQWTCKARQ**RNN--K**MDSLYIAG-RN	ENYQQLA-GKRSK
Volterra	JF502417	B3	.G..............	....	....	I.....S.....E.	N....R..T**K-----**---V....-.D	K..N..P-T..R.
TR-DM	JF502418	B3	.G..............	....	NA	NA	NA	NA
CAEV Cork	M33677	B1	.N..........FM..	................P.	............I..	...**G**.I.....TT.	NK...AP...**DG--**.T.......GKK	...AKTRIIN.K.
It-Pi1	AY265456	B2	.L..........FM..	................P.	NA	NA	NA	NA
Ov496	FJ195346	B2	.L...A......FM..	................QA	....	...**G**.I......T.	NK...AP.W.**GG--M**S.......GKQ	SSHN.R--ST.R.
1GA	AF322109	C	.QVE....S.A..N..	...........Q..Q.--	..V............	I..**S**.LQ.Q.NRS.	RHYVN.RVI**K.D---**-----------	D..ET.P--G.RR
K1514	M60610	A	.G.....TS.REF...	.........V.Q...GP-	H.......QH.....	...**G**.I......T.	NK...A..T**GRKGSQ**R.......-.D	QS.MEAQGKN.RS
kv1772	L06906	A	.G.....TS.REF...	.........V.Q...GP-	........QH.....	...**G**.I......T.	NK....S--**-----Q**R..-....-.D	QS.MEAQGENKRS
SAOMVV	M31646	A	.QV....TS.REF...	.........V.Q...GP-	H.......QH.....	...**G**.I......TD	NK...A..N**SKK--**.R.......-.D	KA.REKNMRNKRS
It-561	AY265455	A	.N.....TS.REF...	...D.....V.Q...GP-	NA	NA	NA	NA
Roccaverano	EU293537	E1	RSM....ESR..FV..	...K...T.M.Q..Q.P-	..I......G.....	L**DAQG**VI....KE.	N.....P...**G.--R**T..V..GA-.R	TG.ETPTIRR.RR
Seui	GQ381130	E2	RSM...KESQ..FV..	...K...T.M.Q..Q.P-	..I......G.....	I.....I....AQK	GH....P.T**KEG--**.T.....G.-KK	AE.AEPSRRRKKR

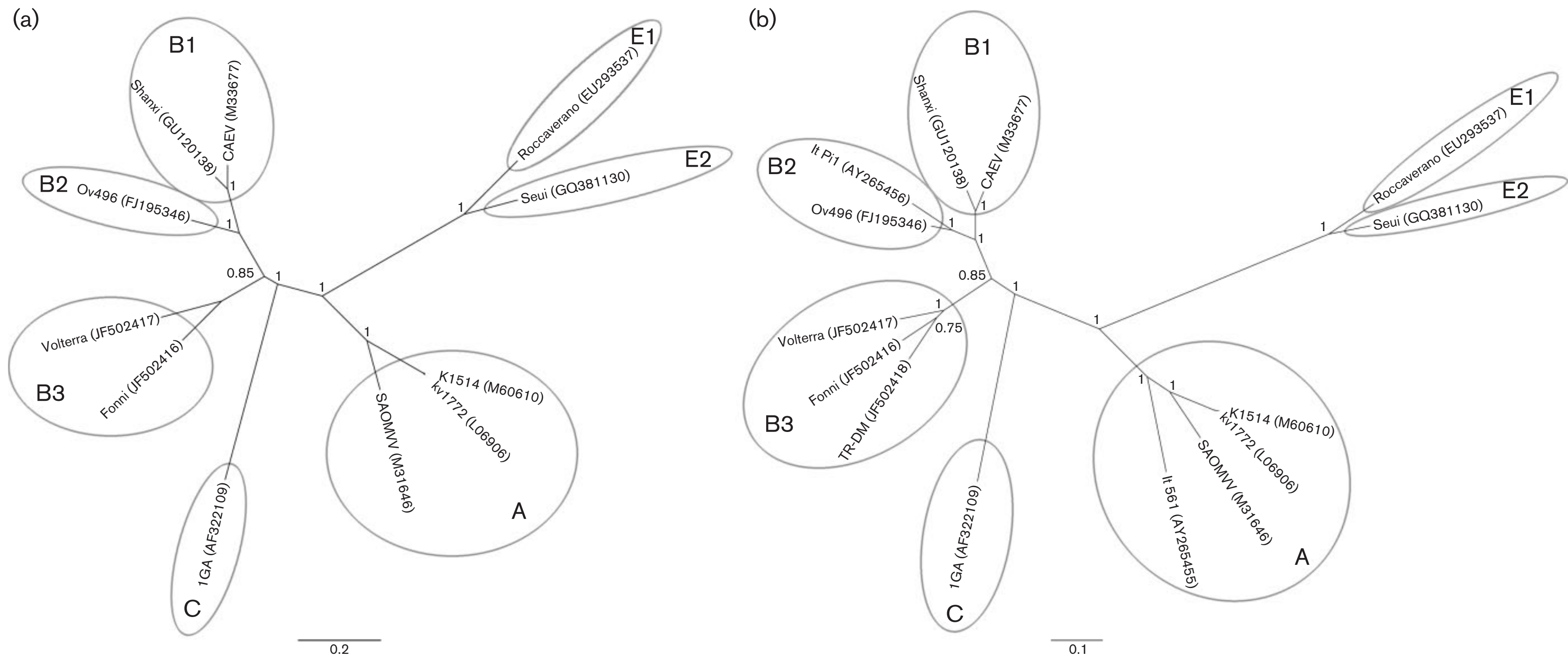

Fig. 1. Phylogenetic tree obtained by (a) concatenating *gag*, *pol* and *env* genes of the main reference SRLV strains and (b) using complete *gag* gene sequences. Posterior probability is shown for each node.